

27 September 2013 | \$10

Science



Curiosity at Gale Crater

SPECIAL SECTION

Curiosity at Gale Crater

INTRODUCTION

- 1475** Analysis of Surface Materials by the Curiosity Mars Rover
J. P. Grotzinger

RESEARCH ARTICLE ABSTRACTS

- 1476** The Petrochemistry of Jake_M: A Martian Mugearite
E. M. Stolper et al.
Soil Diversity and Hydration as Observed by ChemCam at Gale Crater, Mars
P.-Y. Meslin et al.

X-ray Diffraction Results from Mars Science Laboratory: Mineralogy of Rocknest at Gale Crater

D. L. Bish et al.

Curiosity at Gale Crater, Mars: Characterization and Analysis of the Rocknest Sand Shadow
D. F. Blake et al.

Volatile, Isotope, and Organic Analysis of Martian Fines with the Mars Curiosity Rover

L. A. Leshin et al.

>> *Science Podcast*

>> For full text: www.sciencemag.org/extra/curiosity



pages 1430, 1442, & 1457

EDITORIAL

- 1430** Mercury and Health
Marcia McNutt
>> *News package p. 1442; Perspective p. 1457*

NEWS OF THE WEEK

- 1434** A roundup of the week's top stories

NEWS & ANALYSIS

- 1437** Sequester Takes Uneven Bite From Agency Budgets
- 1438** U.S. Carbon Plan Relies on Uncertain Capture Technology
- 1439** Will New Government Overcome 'Symbolically Challenged' Start?
- 1440** DNA Sleuths Track *C. difficile* Infection Routes
- 1441** Zombie Endocrine Disruptors May Threaten Aquatic Life
>> *Science Express Report by S. Qu et al.*

NEWS FOCUS

- 1442** Taming a Mercurial Element
- 1443** With Pact's Completion, the Real Work Begins
>> *Science Podcast*
- 1446** In Minamata, Mercury Still Divides
- 1448** Gold's Dark Side
>> *Editorial p. 1430; Perspective p. 1457*

LETTERS

- 1451** L'Aquila's Aftershocks Shake Scientists
E. Boschi
Low Marks for Education Funding Priorities
J. D. McInerney
Bayes' Confidence
D. A. S. Fraser
Research Funders Should Take the Field
E. Allen et al.

1452 CORRECTIONS AND CLARIFICATIONS

BOOKS ET AL.

- 1453** Experiencing Art
A. P. Shimamura, reviewed by N. Hutton and L. Kelly
- 1454** Behind the Shock Machine
G. Perry, reviewed by R. Abma

EDUCATION FORUM

- 1455** Increasing Persistence of College Students in STEM
M. J. Graham et al.

PERSPECTIVES

- 1457** Global Change and Mercury
D. P. Krabbenhoft and E. M. Sunderland
>> *Editorial p. 1430; News package p. 1442*
- 1458** A Coat of Many Functions
D. Shchukin and H. Möhwald
- 1460** Sources of Antimicrobial Resistance
M. E. J. Woolhouse and M. J. Ward
>> *Report p. 1514*

CONTENTS continued >>

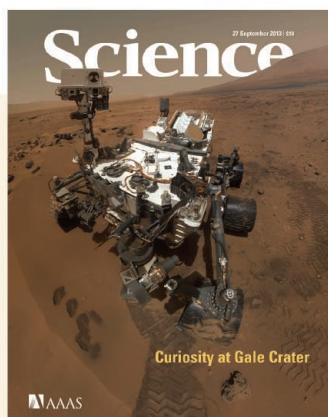
ON THE WEB THIS WEEK

>> Science Podcast

Listen to stories on monitoring mercury in the environment, news from the Curiosity rover, codon bias in bacterial genes, and more.

>> Find More Online

Check out *Science Express*, our podcast, videos, daily news, our research journals, and *Science Careers* at www.sciencemag.org.



COVER

NASA's Mars Science Laboratory rover, Curiosity, at its Rocknest sample-collection site, Gale crater, Mars, on 31 October 2012. This is a mosaic of 59 images acquired by the Mars Hand Lens Imager, a camera mounted at the end of Curiosity's robotic arm (both partly not visible during imaging and cropped out during processing). Four scoop troughs are shown; a fifth was created after the mosaic was obtained. The width of each rover wheel is 40 cm. See page 1475.

Image: NASA/JPL-Caltech/Malin Space Science Systems

DEPARTMENTS

- 1429** This Week in *Science*
- 1431** Editors' Choice
- 1432** *Science* Staff
- 1469** AAAS News & Notes
- 1526** Gordon Research Conferences
- 1537** New Products
- 1538** *Science Careers*

- 1461 A New Route for Growing Large Grains in Metals**
E. M. Taleff and N. A. Pedrazas
 >> Report p. 1500
- 1462 Some Monocytes Got Rhythm**
D. Druzd and C. Scheiermann
 >> Research Article p. 1483
- 1464 Small Volumes Create Super(elastic) Effects**
K. T. Faber
 >> Report p. 1505
- 1465 Stalemate in the Golgi Battle**
B. Morriswood and G. Warren

SCIENCE PRIZE ESSAY

- 1467 Students Propose Genetic Solutions to Societal Problems**
S. Wick et al.

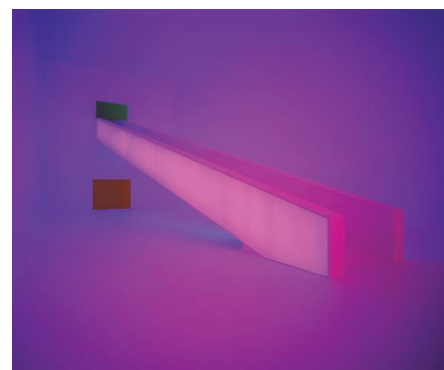
RESEARCH ARTICLES

- 1478 Electromagnetic Energy Conversion at Reconnection Fronts**
V. Angelopoulos et al.
 Data from various satellites in Earth's magnetotail clarify where and how electromagnetic energy conversion occurs.
- 1483 Circadian Gene *Bmal1* Regulates Diurnal Oscillations of Ly6C^{hi} Inflammatory Monocytes**
K. D. Nguyen et al.
 The clock protein *Bmal1* regulates daily changes in white blood cell trafficking and susceptibility to inflammation in mice.
 >> Perspective p. 1462

REPORTS

- 1489 In Situ Observations of Interstellar Plasma with Voyager 1**
D. A. Gurnett et al.
 Electron densities detected by Voyager 1 show that the spacecraft is in the interstellar plasma.
- 1492 Distances, Luminosities, and Temperatures of the Coldest Known Substellar Objects**
T. J. Dupuy and A. L. Kraus
 Observations with the Spitzer Space Telescope strengthen the link between the coolest brown dwarfs and gas-giant exoplanets.
- 1496 Observation of Dirac Node Formation and Mass Acquisition in a Topological Crystalline Insulator**
Y. Okada et al.
 Scanning tunneling spectroscopy of $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ in a magnetic field reveals two types of Dirac fermions.

- 1500 Abnormal Grain Growth Induced by Cyclic Heat Treatment**
T. Omori et al.
 Thermal cycling of a copper-based shape-memory alloy leads to abnormal grain growth and very large grains.
 >> Perspective p. 1461
- 1502 Cation Intercalation and High Volumetric Capacitance of Two-Dimensional Titanium Carbide**
M. R. Lukatskaya et al.
 The layered material Ti_3C_2 can intercalate much larger cations than Li^+ , allowing for energy storage applications.
- 1505 Shape Memory and Superelastic Ceramics at Small Scales**
A. Lai et al.
 Fine-scale shape memory ceramics are capable of many actuation cycles to strains up to 7%.
 >> Perspective p. 1464
- 1508 Near-Complete Extinction of Native Small Mammal Fauna 25 Years After Forest Fragmentation**
L. Gibson et al.
 The rapid loss of native mammals from isolated Thai forests suggests that forest fragments cannot maintain biodiversity.
- 1511 Safeguards for Cell Cooperation in Mouse Embryogenesis Shown by Genome-Wide Cheater Screen**
M. Dejosez et al.
 During embryogenesis, a network of genes centered on *p53*, *topoisomerase 1*, and olfactory receptors helps to ensure cell cooperation.
- 1514 Distinguishable Epidemics of Multidrug-Resistant *Salmonella* Typhimurium DT104 in Different Hosts**
A. E. Mather et al.
 Antibiotic resistance travels in independent epidemics in humans and their livestock.
 >> Perspective p. 1460
- 1517 The Inhibitory Circuit Architecture of the Lateral Hypothalamus Orchestrates Feeding**
J. H. Jennings et al.
 A specific brain circuit drives the consumption of highly palatable food, even when energy needs are satisfied.
- 1521 Cocaine Disinhibits Dopamine Neurons by Potentiation of GABA Transmission in the Ventral Tegmental Area**
C. Bocklisch et al.
 Changes in specific neuronal circuits suggest that drug-evoked synaptic plasticity facilitates drug-adaptive behavior.



page 1453



pages 1460 & 1514



pages 1461 & 1500

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2013 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$149 (\$74 allocated to subscription). Domestic institutional subscription (51 issues): \$990; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. Postmaster: Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. Single-copy sales: \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$30.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8075. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.



The Overeating Connection

Obesity has become a major global health problem. Working in mice, **Jennings *et al.*** (p. 1517) identified an important brain circuit within the lateral hypothalamus that modulates food intake. The findings reveal the neuronal connections that drive the consumption of highly palatable food even when energy needs are satisfied. Inhibition of this circuit suppressed feeding.

Observing Earth's Magnetotail

Magnetic reconnection is a process that converts magnetic energy to kinetic energy, thermal energy, and particle acceleration. The process operates in Earth's magnetotail, the narrow and elongated region of the magnetosphere that extends away from the Sun, and is believed to power Earth's auroras and other space physics phenomena. **Angelopoulos *et al.*** (p. 1478) present an observational study of energy conversion in the magnetotail and the associated transport of magnetic flux during a geomagnetic substorm.

Shape Memory Ceramics

Shape memory materials convert heat into strain and vice versa. These materials are often made from metal alloys, which can generate large stresses with small shape changes. Ceramics have certain characteristics that underlie shape memory transformation, but generally crack under strain. **Lai *et al.*** (p. 1505; see the Perspective by **Faber**) introduce a modified ceramic structure with limited crystal grains that can withstand comparable cyclic strains to shape memory metals.

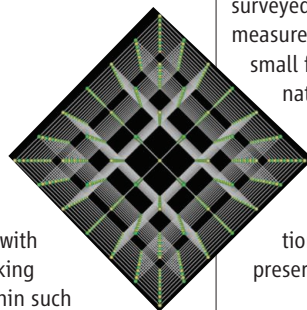
Diurnal Immunology

Like most organ systems in the body, several components of the immune system appear to be regulated in a diurnal manner. However, the cell populations affected, the underlying molecular mechanisms controlling these processes, and the functional consequences of such regulation are poorly understood. Now, **Nguyen *et al.*** (p. 1483, published online 22 August;

see the Perspective by **Druzd and Scheiermann**) have found that trafficking of monocytes to sites of inflammation is regulated in a diurnal manner in mice. Mice harboring a myeloid cell-specific deletion in the clock protein, **BMAL1**, showed reduced fitness in response to both acute and chronic inflammation.

Half-Massless

Certain materials, such as topological crystalline insulators (TCIs), host robust surface states that have a Dirac (graphene-like) dispersion associated with massless carriers; the breaking of protective symmetry within such materials should cause the carriers to acquire mass. **Okada *et al.*** (p. 1496, published online 29 August) used scanning tunneling microscopy to map out the energies of the electronic levels of the TCI $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ as a function of the strength of an external magnetic field. The massless Dirac fermions coexisted with massive ones, presumably as a consequence of a distortion of the crystalline structure affecting only one of the two mirror symmetries.



Making the Grain

Most metals contain a large number of ordered crystalline regions that are separated by disordered grain boundaries. If the material is annealed at elevated temperatures, the larger grains will grow uniformly at the expense of the smaller ones. This process slows down over time, making it hard to create very large

grains. Abnormal grain growth, in which a few of the crystalline regions grow much faster and larger than the others, can occur if the material is put through a complex annealing process involving straining of the samples. **Omori *et al.*** (p. 1500; see the Perspective by **Taleff and Pedrazas**) find that a much simpler and shorter annealing process can trigger abnormal grain growth in copper-based shape-memory alloys. Thermal cycling between a high-temperature single-phase region and a lower-temperature two-phase region generated dislocations at low temperatures and grain growth on heating. Because this method does not require external straining of the sample, it is not limited to thin sheets or wires.

Futile Forest Fragments

Most of the planet's terrestrial biodiversity is found in tropical forests, but much of this critical habitat now persists as fragmented patches surrounded by agriculture. Smaller forest patches sustain fewer species than larger patches or contiguous forest. However, the numbers of species that will disappear from a forest fragment—and the rate of species loss—remain poorly understood. **Gibson *et al.*** (p. 1508) surveyed islands in a reservoir in Thailand to measure the rate of loss of small mammals from small forest fragments. Collapse of the entire native community (up to 12 species) from 16 forest fragments was observed after 25 years of isolation. Thus, small forest fragments hold little value for mammalian biodiversity, and conservation efforts should instead focus on the preservation of large forest expanses.

Sourcing Antibiotic Resistance

It is widely assumed that antibiotic resistance in farm animals contributes in a major way to antibiotic resistance in humans. **Mather *et al.*** (p. 1514, published online 12 September; see the Perspective by **Woolhouse and Ward**) analyzed hundreds of genome sequences from *Salmonella* isolates collected from both livestock and patients in Scotland between 1990 and 2004. The relative contributions of animal-derived and human-derived sources of infection were quantified and the phylogenetic diversity of resistance profiles was matched with bacterial phylogenies. The results suggest that most human infections are caught from other humans rather than from livestock and that humans harbor a greater diversity of antibiotic resistance.

Additional summaries

Finally Out

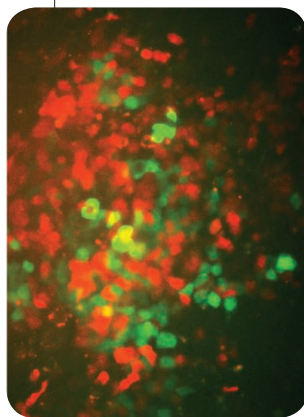
Last summer, it was not clear if the Voyager 1 spacecraft had finally crossed the heliopause—the boundary between the heliosphere and interstellar space. **Gurnett *et al.*** (p. 1489, published online 12 September) present results from the Plasma Wave instrument on Voyager 1 that provide evidence that the spacecraft was in the interstellar plasma during two periods, October to November 2012 and April to May 2013, and very likely in the interstellar plasma continuously since the series of boundary crossings that occurred in July to August 2012.

Assessing Brown Dwarfs

The last 2 years have seen the detection of dozens of very cold brown dwarfs. At temperatures around 300 to 500 kelvin, brown dwarfs are expected to have masses comparable to those of gas-giant planets, but because their distances are unknown, it has not been possible to estimate their masses. **Dupuy and Kraus** (p. 1492, published online 5 September) used data from the Spitzer Space Telescope to measure accurate distances to very cold brown dwarfs, which allowed them to determine the dwarfs' luminosities, temperatures, and masses. The results strengthen the connection between the coolest brown dwarfs and gas-giant exoplanets.

Keeping Cells Cooperating

Multicellular organisms have certain advantages over those that are single-celled. To evolve, however, they must surmount a persistent challenge: ensuring that their constituent cells cooperate with one another rather than “cheat” or compete with each other for resources. **Dejosez *et al.*** (p. 1511, published online 12 September) performed a genome-wide screen in induced pluripotent stem cells to search for genes that



promote cell cooperation. A number of genes were identified of which knockdown allowed competitive behavior to dominate. These genes formed a network centered on p53, topoisomerase 1, and olfactory receptors. Thus, a genetic mechanism may promote cooperation in the developing embryo.

Drugs, Dopamine, and Disinhibition

Drugs often change the neuronal circuitry in the brain and thereby cause a long-lasting change in behavior. Using a wide range of in vivo and in vitro techniques in mice, **Bocklisch *et al.*** (p. 1521) observed that cocaine profoundly altered dopamine neuron function and that drug-evoked synaptic plasticity in a specific set of neurons represents a crucial step in circuit remodeling.

Toward Titanium Carbide Batteries

Many batteries and capacitors make use of lithium intercalation as a means of storing and transporting charge. Lithium is commonly used because it offers the best energy density, but also because there are difficulties in storing larger cations without disrupting the crystal structure of the host. **Lukatskaya *et al.*** (p. 1502) developed a series of MX compounds, where M represents a transition metal and X is carbon or nitrogen. The compound Ti₃C₂ forms a two dimensional layered structure, which is capable of accommodating a wide range of cations, including multivalent ones, either spontaneously or electrochemically.

Mercury and Health

THIS OCTOBER, NATIONS WILL GATHER IN JAPAN TO SIGN THE MINAMATA CONVENTION, A TREATY TO address the toxic effects of mercury in the environment. The agreement will become binding once ratified by at least 50 nations. The convention is timely and welcome in that it places controls and limitations on products, processes, and industries that increase the level of exposure of people and the environment to mercury, a naturally occurring element. Mercury bioaccumulates in the form of methylmercury, a powerful neurotoxin that can affect wild-life, domestic animals, and humans alike. Symptoms of mercury poisoning can range from numbness in the hands and feet and muscle weakness in mild cases, to insanity and death.

This convention is named after the Japanese city where the release of methylmercury in the wastewater from a chemical factory from 1932 to 1968 polluted the local seafood, which then caused severe mercury poisoning in thousands of local residents dependent on that resource. Minamata was a case where science provided the evidence connecting the cause (effluent discharge) with the effect (neurologically diseased patients). Seafloor sediment samples recovered from the bay offshore of Minamata showed

that the concentration of methylmercury contamination was highest near the industrial outflow to the ocean and decreased from there. Samples of local seafood and shellfish were laden with mercury. Hair samples from the afflicted population showed highly toxic amounts of the element. Local cats, consumers of the scrap seafood from their owners' tables, were the first to die. Ultimately, the industrial discharge ceased, and the responsible party was ordered to compensate the victims.

Critics of the Minamata Convention believe that it does not go far enough. For example, in 2010 the top two sources of mercury pollution were artisanal gold mining (727 tons) and coal-fired power plants (475 tons).^{*} As many as 15 million adults and children live in communities at risk from mercury pollution from artisanal gold mining, primarily in Africa and South America. Mercury from coal-

fired power plants can be transported through the atmosphere far from the source, deposited in aquatic systems, and then converted to methylmercury. The population at risk may have had no voice in the decision to install the coal-fired facility. The treaty stops short of specifying goals or target dates for action plans for reducing mercury release from mining, nor does it require refitting of existing coal-fired power plants to reduce mercury emissions.

One roadblock standing in the way of stronger controls on mercury in the environment, better national action plans, and perhaps even swifter ratification of the Minamata Convention is that many environmental and health aspects of mercury are poorly known,[†] except in the most acute cases of mercury poisoning. Science can help by linking research on mercury's behavior in the environment (see the Perspective by Krabbenhoft and Sunderland on p. 1457) with studies of its public health impacts. Research teams need to include environmental toxicologists who understand the biochemistry of toxic contaminants, ecologists who trace their bioaccumulation through the food chain, physicians who understand the effects of chronic and acute exposure on long-lived species such as us, and public health specialists who can extract patterns from large populations. What geographic areas are most at risk? Which population demographics are particularly vulnerable? What symptoms should we look for? What other factors might confound their identification? Even at sublethal levels, does exposure to mercury have impacts on the development of young children? Is there a level of mercury consumption that is perfectly safe?

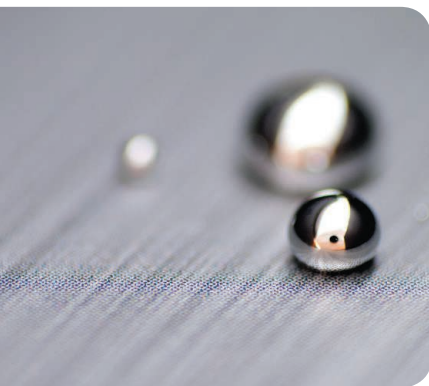
Such investigations cross the boundaries of government agencies and of university schools and departments. Funding the many investigators could be a challenge. But with the number of coal-fired power plants projected to rise globally,[‡] tracing the connections between mercury pollution and health must be a priority.

— Marcia McNutt

^{*}www.unep.org/PDF/PressReleases/GlobalMercuryAssessment2013.pdf. [†]For examples of some scientific frontiers, see www.mercury2013.com. [‡]www.wri.org/publication/global-coal-risk-assessment.



Marcia McNutt is Editor-in-Chief of *Science*.



CELL SIGNALING

Fueling Reproduction

New studies in yeast may improve our understanding of human metabolic disorders. Clement *et al.* characterized regulation of the heterotrimeric guanine nucleotide-binding protein (G protein) alpha subunit Gpa1 in yeast. Gpa1 is part of the G protein that facilitates signaling by the receptor for mating pheromone. Clement *et al.* report that Gpa1 provides a molecular link to signaling pathways that monitor nutrient availability. The same enzymes that regulated phosphorylation of the yeast homolog of AMPK (adenosine monophosphate-activated protein kinase, an enzyme critical to energy sensing in mammals) also regulated phosphorylation of Gpa1 when cells were deprived of glucose. Such phosphorylation-modulated activity of Gpa1 thus couples mating efficiency to nutrient availability. These signaling pathways are highly conserved, and thus these results suggest possible crosstalk between nutrient and energy-sensing pathways and G protein-coupled receptor-regulated processes in higher organisms as well. Schmidt provides context in a Perspective. — LBR

Sci. Signal. **6**, 10.1126/scisignal.2004143; 10.1126/scisignal.2004589 (2013).

GEOCHEMISTRY

Banding Together

Speleothems (stalagmites, stalagmites, and related secondary mineral deposits formed in caves) are commonly dated by counting the growth bands they contain and assuming that those bands are annual markers. How good is this assumption? Shen *et al.* present results of a study of a 300-year-old laminated stalagmite from Xianren Cave, China, using high-precision radiometric



²³⁰Th dating with a 2σ precision of half a year. They find that the layers of the speleothem do not always deposit annually, as is commonly assumed, and that band counting can under- or overcount elapsed time by several years or more per decade in some cases. Thus, caution needs to be applied when using band counting as a dating tool for speleothems, particularly because even



ANIMAL BEHAVIOR

Queenless Altruism

The activity of honey bee (*Apis mellifera*) colonies is centered around a single reproductive female, the queen. Sterile worker females populate the majority of the hive, with the remainder composed of a small number of males. Worker tasks vary and include foraging, care for the queen and young, and hive construction and defense, with tasks largely corresponding to age (younger bees care for young, whereas older ones forage and defend the hive). In a hive that has lost its queen, some workers start to lay eggs, but their offspring will all be male. Kin selection theory suggests that worker altruism in queenless colonies will be reduced because the workers are less related to the sibling's progeny than that of the queen. Naeger *et al.* examine altruism and social organization in a queenless hive. They find that egg-laying workers were as likely to forage or participate in hive defense as non-egg layers, who have less developed ovaries. Further, reproductive workers were metabolically invested in brood food and wax production. This results in a hive with decreased task specialization, so that bees that forage also care for the brood and maintain the structure of the hive; a case more similar to what is seen in solitary social bees. This work supports kin selection models in that reproductive conflict is increased in queenless colonies but altruism is still present. Queenless workers participate in their own reproduction but also display altruistic behaviors that benefit the colony. — BAP

Curr. Biol. **23**, 1574 (2013).

small errors in dating can affect the interpretation of some rapid climate change events; high-precision absolute-dated radiometric chronologies should be used when possible. — HJS

Sci. Rep. **3**, 2633 (2013).

PHYSICS

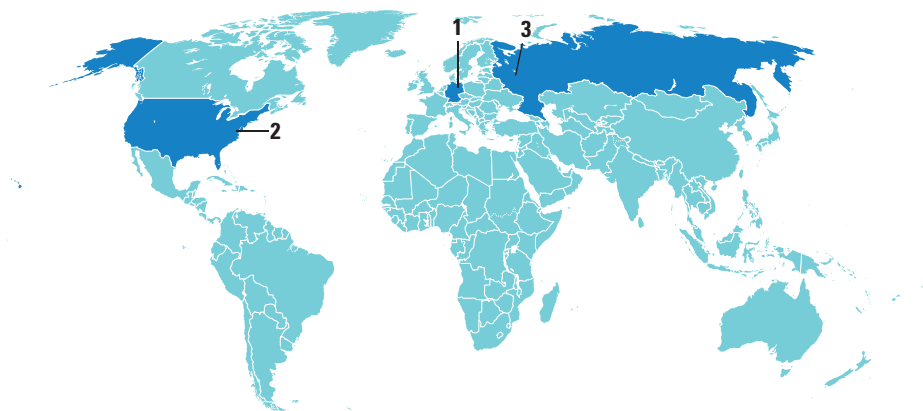
Tricky Anomaly

A quantum point contact is a narrow conducting channel that can be shaped by applying variable voltage to electrodes placed above a two-dimensional electron gas in semiconductor nanostructures. As a function of the voltage, the conductance of the contact exhibits equally spaced plateaus corresponding to sequential openings of transport channels; however, an additional feature that appears at the conductance value

of roughly 0.7 of the step still lacks a detailed theoretical explanation. Bauer *et al.* argue that it is a consequence of the structure of the local density of states. They developed a one-dimensional tight-binding model—in which a singularity in the density of states resulted in enhanced electronic interactions—that was able to account for data taken in varying experimental conditions. Iqbal *et al.* ascribe the feature to localized spins and the Kondo effect. They performed their experiments on quantum point contacts of varying length and found that the conductance of the anomaly varied periodically with the length; in their model, the number of localized states increased with the length, explaining the periodicity. Both models offer considerable and complementary insights into the problem. — JS

Nature **501**, 73; 79 (2013).

AROUND THE WORLD



Berlin 1

A Win for German Science?

German Chancellor Angela Merkel, who started her career as a quantum chemist, and her party, the center-right Christian Democrats (CDU), were the big winners in Sunday's elections, with 41.5% of the vote—far ahead of their main rivals, the Social Democrats (SPD), who received 25.7%.

The victory presents Merkel with a quandary, however, because her favored coalition partner, the Free Democrats, failed to reach the 5% cutoff for representation in the German parliament. That



Winning smile. German Chancellor Angela Merkel was reelected Sunday.

means she has to form a coalition with one of two center-left parties, the SPD or the eco-oriented Greens. The negotiations are expected to take weeks.

Germany's "*Energiewende*," an effort to transition away from nuclear and fossil fuels toward renewables, is likely to continue no matter what coalition forms. All the parties have ambitious carbon dioxide reduction

goals in their platforms. Both CDU and SPD see efficient coal and gas as an acceptable bridge technology. The Green Party wants to block any new coal-fired plants.

Many research leaders hope the new government will pass a constitutional amendment allowing the federal government to finance universities directly.

<http://scim.ag/Merkelwin>

Bethesda, Maryland 2

NIH's Alzheimer's Gamble

The National Institutes of Health (NIH) announced last week that it will give \$33 million to yet another clinical trial of a drug that targets amyloid plaques—the hallmark protein tangles that clog brain cells in people with Alzheimer's disease.

So far, such drugs have failed in clinical trials, but some scientists believe this is because they were administered too late, after brain damage is irreversible. Lead researchers Eric Reiman and Pierre Tariot of the Banner Alzheimer's Institute in Phoenix plan to give a yet-to-be-identified anti-amyloid drug to 650 participants in their 60s and 70s who carry two copies of the *APOE4* gene—a genetic double whammy that confers a 10-fold increased risk of developing Alzheimer's late in life. Roughly a third will likely not have much amyloid in their brains yet, allowing the researchers to track whether the drug affects its accumulation, Reiman says.

Given that no anti-amyloid drugs have yet shown clinical efficacy, the agency is "taking a gamble" by using the lion's share of its Alzheimer's research budget for the study, says Gary Landreth, a neurologist who studies the disease at Case Western Reserve University in Cleveland, Ohio. <http://scim.ag/NIHAlzheimers>



Shakeup. The Russian Academy of Sciences will be merged with two other academies.

Moscow 3

RAS Merger Goes Forward

In June, when Russian legislators agreed to delay finalizing a bill that would strip the Russian Academy of Sciences (RAS) of control of its budget and real estate holdings and merge it with two other academies, scientists saw a chance to press their case for keeping the august body intact (*Science*, 12 July, p. 114). But more than 2 months of lobbying and protests were in vain: Russia's lower house of parliament has approved a measure that will radically transform the 289-year-old academy.

Under the bill, expected to be approved by parliament's upper house and then signed into law by President Vladimir Putin this week, a new Agency for RAS Scientific Institutions, directed by the academy's president, Vladimir Fortov, will manage the budget and assets of the merged academies. Researchers worry that Fortov will be only a figurehead and that officials inimical to RAS will pull the strings. "It's like being a member of an orchestra whose conductor is not a musician but a bureaucrat," fumes Vladimir Zakharov, a physicist at RAS's P. N. Lebedev Physical Institute in Moscow.

NEWSMAKERS

Smithsonian Secretary To Step Down

G. Wayne Clough, 72, announced last week that he will step down as head of the U.S.



Smithsonian Institution in October 2014. Clough, who started as secretary in July 2008, says his focus from the start was on "restoring the vitality and forward-looking ethic" of the organization; concerned about the Smithsonian's reputation as "the nation's attic," which suggested that most of

its 137 million specimens and objects were hidden away gathering dust, he conducted vigorous outreach to the U.S. Congress, potential donors, and the public, and used the Internet to expand access to collections and interactive programs. Clough also laid the groundwork for the Smithsonian's first national capital campaign, which has already netted \$893 million in private contributions since 2008.

Clough came to the Smithsonian promising to stay 5 years, but didn't want to commit to another five. "You can't do [this job] at 80 or 90%, you have to do it at 110%." He insists that he's not retiring, but plans to spend more time with family and on book projects. The Board of Regents that oversees the Smithsonian says it will be launching an international search for Clough's successor. <http://scim.ag/Cloughend>

FINDINGS

Naps Nurture Young Brains

With many preschools boasting an expansive curriculum, naps sometimes fall by the wayside. Psychologist Rebecca Spencer of the University of Massachusetts, Amherst, decided to find out if that was a problem for the 3- to 5-year-old set.

She and her colleagues taught young children a memory game that involved finding matching picture cards. On one day, kids napped after learning the game; on another, they were kept awake. They played the game after napping (or not) and again the next morning.

The "habitual" nappers—those accustomed to taking naps—remembered about 15% fewer cards if deprived of a nap, the team reported online this week in the *Proceedings of the National Academy of Sciences*. And the sleep-deprived nappers never caught up: When playing the game the next day, they still performed more poorly than when they napped. "They need



Making memories. Naps help kids (such as the study author's daughter) remember what they learned.

that sleep close to learning" for it to take root, Spencer says.

Spencer and her students also studied the brain activity of 14 children in their sleep laboratory. They found "sleep spindles," short bursts of activity associated with memory processing. "If they need the nap," Spencer says, "they should get the nap." <http://scim.ag/napbrains>

Fascinatin' Rhythms

Circadian clocks keep animals in step with Earth's daily solar cycle. But many animals also stay in sync with other oscillations, such as tides and the phases of the moon.

THEY SAID IT

"Cynically, this feels like a ploy to boost Google's mystique. Optimistically, it feels like another one of Google's fickle interests."

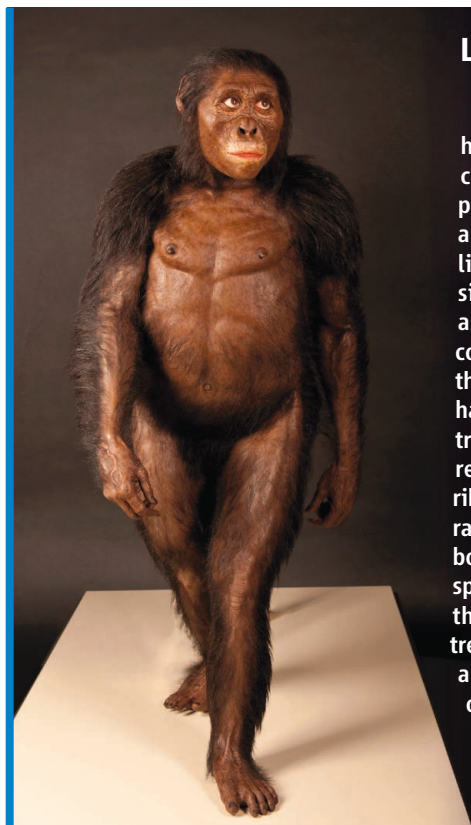
—Caleb Garling of the *San Francisco Chronicle*, commenting last week on Google's creation of Calico, a biotech firm with vague plans to address aging and its associated diseases.

Two papers published this week in *Current Biology* and *Cell Reports* show that these alternate rhythms are driven by molecular clocks that are independent of the one driving circadian activities.

Researchers in the United Kingdom studied both circadian and tidal cycles in the speckled sea louse, the marine crustacean *Eurydice pulchra*. The louse changes the color pattern on its shell on a daily cycle, but is also more active at high tide, while swimming less at low tide. The researchers showed that by changing the

Lucy Gets a New Look

The 3.2-million-year-old hominin Lucy has had a makeover, thanks to newly discovered fossils. The reconstruction displays a trimmer figure with a distinct neck, a narrower waistline, and arched foot. Earlier reconstructions, relying on scanty fossil rib bones and living African apes such as chimpanzees and gorillas, gave her a cone-shaped thorax and potbelly, implying that her species, *Australopithecus afarensis*, had retained adaptations for moving in the trees like chimps. But in the past few years, researchers have found curved *A. afarensis* ribs, which translates to a barrel-shaped thorax like modern humans, and an arched foot bone, which suggests that Lucy and her kin spent plenty of time on the ground, although they probably still climbed and slept in trees. The reconstruction, overseen by paleo-anthropologist Yohannes Haile-Selassie of the Cleveland Museum of Natural History and created by artist John Gurche, was unveiled on 20 September at Ohio's Cleveland Museum of Natural History.



NOTED

>NASA's **Deep Impact mission** was officially declared dead last week, after more than 8 years in space, 7.6 billion kilometers traveled, and a close-up view of two comets—slamming an impactor into one and flying by a second during the probe's long, successful life. Its last signal was received on 8 August; NASA declared the mission over last week.

CREDITS (TOP TO BOTTOM): IMAGE COURTESY OF REBECCA SPENCER; RECONSTRUCTION BY JOHN GURCHE, COURTESY OF THE CLEVELAND MUSEUM OF NATURAL HISTORY

BY THE NUMBERS

33% Reduction in the annual number of new HIV infections worldwide from 2001 to 2012, according to a UNAIDS update released on 23 September.

\$1.4 trillion Economic rewards of the estimated 2.3 billion tons of world crops that could be grown with better land management, according to a U.N.-backed report published on 24 September by the Economics of Land Degradation Initiative.

\$500 million Amount Oregon Health & Science University officials have pledged to raise for cancer research in the next 2 years, to receive a matching donation from philanthropists Phil and Penny Knight.

>>FINDINGS

animals' exposure to light or vibration (to simulate tides), they could manipulate the two cycles independently of each other, settling an old debate about whether the



Different beat. The marine worm *Platynereis dumerilii* matures in sync with the phases of the moon.

tidal clock was simply a subset of the circadian one.

Austrian researchers studying the marine bristle worm *Platynereis dumerilii* found similar evidence that the lunar-driven clock that controls the animals' maturation—they respond to changes in moonlight—is separate from the circadian one. It's likely, the researchers say, that many animals carry multiple clocks. <http://scim.ag/tidalclock>

Multiple Lineages for MERS

A new genetic analysis of Middle East respiratory syndrome (MERS) reveals that several varieties of the deadly virus are circulating in Saudi Arabia, leading some scientists to believe it reached humans from

multiple sources. The origin of the virus, which has killed 58 people since 2012, is still unclear.

Virologist Paul Kellam of the Wellcome Trust Sanger Institute in Hinxton, U.K., and colleagues sequenced the genomes of virus samples from 21 patients in Saudi Arabia and constructed a genetic tree based on differences between the samples. The analysis, published online last week in *The Lancet*, identifies at least three distinct lineages of MERS. The variation among genomes collected within days of each other suggests that either the virus has evolved while moving through a large asymptomatic population, or that it diversified in animals and infected humans on several occasions. The authors favor this second interpretation, which highlights the need to identify an animal source and limit its contact with humans, Kellam says. Other researchers say both scenarios are equally plausible from the available data. <http://scim.ag/MERSgenome>

Science LIVE

Join us on Thursday, 3 October, at 3 p.m. EDT for a live chat on "Rocknest" and the latest findings from the Mars Curiosity rover. <http://scim.ag/science-live>

Random Sample

String Theory Goes Viral

"Is string theory right? Is it just fantasy?" sings Tim Blais in "Bohemian Gravity," the a cappella song parody that has garnered more than 1 million hits on YouTube, earned a plethora of blog mentions, and won the 23-year-old musician his 15 minutes of online fame. A take on the 1975 Queen rock aria, Blais's creation features complex musical arrangements and video that he edited over the last year while he worked to complete a master's degree in physics at McGill University in Montreal.

A singer, piano player, and composer, Blais wrote his first science-themed YouTube foray, "Rolling in the Higgs"—inspired by the soulful song by Adele—during the live-streamed announcement of the Higgs boson discovery last year. Having received his degree, Blais says he was looking forward to leaving science for a while to focus on his first love: performing music. "But now, because of the video, people are encouraging me to do a lot more science-based music," he says.

What do prominent critics of string theory think? "A fabulous bit of '80s nostalgia," was how Lee Smolin of the Waterloo, Canada-based Perimeter Institute put it. "I'm not in the mood to analyze the scientific



Oh mamma mia mamma mia. "Such a sea of particles! A tachyon, with a dilaton and gravity."

arguments," said Peter Woit of Columbia University, but he calls the video "fantastically good."

The recognition most meaningful to Blais? Mention by Queen guitarist Brian May, an astrophysicist who is now chancellor emeritus at Liverpool John Moores University in the United Kingdom. "Is that ME at the top of Brian May's website? I think I just won at life," Blais wrote on Facebook.



U.S. RESEARCH SPENDING

Sequester Takes Uneven Bite From Agency Budgets

For nearly a year now, U.S. research leaders have been issuing dire warnings about layoffs and shuttered labs in the wake of the 5% budget cut, known as the sequester, that hit most federal research agencies in 2013. But as the U.S. fiscal year enters its final week, hard data on how the sequester is affecting the country's scientific enterprise remain scarce.

Several university deans and government laboratory directors report that its bite so far has been less severe than the bark, and the sequester's impact also varies by agency. Last week, Francis Collins, the director of the National Institutes of Health (NIH), announced that NIH would make 650 fewer new competitive awards in 2013, a 7% drop. In contrast, a spokeswoman for the National Science Foundation (NSF) says that, "based on an initial review, we're not seeing any noteworthy changes in the year-to-date numbers."

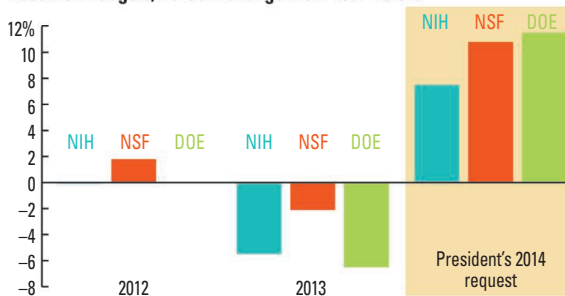
The sequester is a mandatory cut triggered by a 10-year budget agreement struck in 2011 that went bad. And agency officials are already deeply worried about the consequences of a continued squeeze on research budgets. "While NSF was able to largely mitigate the impact of sequestration [this year] ... we remain deeply concerned ... should constrained funding levels continue into [2014] and beyond," NSF acting Director Cora Marrett told *Science*. In July, Collins warned biomedical researchers at a rally hosted by

Johns Hopkins University that sequestration is "putting an entire generation of U.S. scientists and our nation at risk."

Here's a look at how three key agencies have handled this year's sequester, which went into effect in March, and its effect to date on the researchers they fund.

NIH: To trim its spending by 5.5%, to \$29.15 billion, NIH took aim at new grants.

Research Budgets, Percent Change From Year Before



The tally. This year, NSF has weathered the sequester better than DOE and NIH. But the cuts have left all agencies far below their 2014 budget requests. One consequence is fewer NIH grants (right).

Collins expects that only about 15% of proposals reviewed this year will receive funding, down from 18% in 2012. It is a historic low for the agency, which has seen rates tumble from a peak of 37% in 2001.

Ongoing grants are also being trimmed, by an average of 4.7%. And some NIH insti-

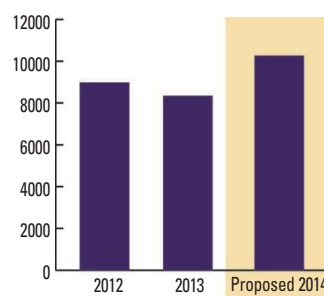
Worried. Scientists rally against the sequester in Washington, D.C., earlier this year.

tutes made deeper cuts in centers, contracts, and other large programs to help shore up investigator-initiated grants. Some clinical trials for AIDS and influenza vaccines were slashed 20%, says Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, which may mean enrolling fewer patients. The base award for the iconic Framingham Heart Study, for example, was reduced by 40%, or \$10 million. Even with the money from that and other cuts, however, the National Heart, Lung, and Blood Institute decided for the first time to offer short-term bridge funding to applicants who need buoying while they await word from NIH on a revised proposal.

Some universities and academic medical centers are reporting a drop in first-year graduate enrollment—13% in the biomedical sciences at Duke, says Chancellor of Health Affairs Victor Dzau, and 11% across all graduate programs at Vanderbilt University, says Graduate School Dean Dennis Hall. Collins stands by his prediction from last spring that 20,000 people on NIH-funded projects would lose their jobs due to the sequester, although NIH has no such unemployment data. "If nothing changes, ultimately that's going to have to be what happens," he says.

On the other hand, the University of Washington in Seattle hasn't trimmed the size of its entering graduate class, says Mary Lidstrom, vice provost for research, and faculty requests for bridge funding actually

New NIH Grants



dropped this summer. Likewise, the dire predictions of 350 staffers being pink-slipped and 50 labs shuttered at the University of Pittsburgh haven't materialized because federal funding exceeded projections and the university used bridge funding to keep many labs afloat, explains Jeremy Berg, associate

senior vice chancellor for science strategy and planning in the health sciences.

NSF: The science foundation enjoys several advantages over NIH that allowed it to cushion the impact of this year's sequester on the research community.

The biggest was that Congress increased NSF's 2013 budget by roughly 3% before the sequester kicked in. "We had a better starting point," says one NSF official. That bump-up meant a net budget reduction of only 2.1% from 2012, to \$6.88 billion (see graphic, p. 1437).

The second advantage stems from the fact that roughly two-thirds of NSF grantees get their entire 3-year award up front in what's called a standard grant. In contrast, only the first year of NIH's typical 4-year award goes out the door to principal investigators, who get the rest of what's called a continuing grant in yearly increments that may fluctuate.

Why does that matter? When faced with a sudden budget crunch, NSF can hold success rates steady by making slightly fewer standard grants and handing out more continuing

"[Sequestration is] putting an entire generation of U.S. scientists and our nation at risk."

—FRANCIS COLLINS,
NIH

grants. It's a short-term solution, to be sure, but it helped NSF keep the overall number of new awards this year close to 2012 levels. (NSF officials say they're still tallying up the final numbers.)

NSF sheltered favorite areas like its graduate research fellowships, advanced manufacturing, and cybersecurity research, as well as an interdisciplinary research initiative launched by its former director, Subra Suresh, who left in March to become president of Carnegie Mellon University. Large construction projects also escaped the ax this year, as NSF followed a White House budget directive that, as one NSF official put it, says, "Don't do something now to save money if it's going to make things worse next year."

In contrast, some disciplines have suffered disproportionate cuts. The budgets of NSF's physics and mathematics divisions shrank by 9.6% and 7.8%, respectively, its environmental biology program has dropped by 6.3%, and both atmospheric and earth sciences were trimmed by 5.3%.

DOE: The Department of Energy's (DOE's) Office of Science, the single biggest funder

of the physical sciences in the United States, managed to weather its 5% sequester, to \$4.63 billion, by eating its seed corn.

Many of the office's 10 national labs, which run large scientific user facilities such as x-ray sources and particle accelerators, had already cut staff in anticipation of a lean year and were able to avoid layoffs and furloughs this year. DOE officials also funded current operations with money from construction projects that were ending. That money normally would have gone for the next construction project.

That change in tactics will make it harder to find sufficient money to launch the next project, however. "Anything that gets started is going to be a dead lift from zero [construction funding], and that's a tough challenge even in the best of times," says Thom Mason, director of the Oak Ridge National Laboratory in Tennessee. Without the cushion of construction money to soften the blow, he adds, future cuts will require DOE to shut down facilities. "The next time it will be a disaster," warns Chi-Chang Kao, director of SLAC National Accelerator Laboratory in Menlo Park, California.

—ADRIAN CHO, JOCELYN KAISER,
AND JEFFREY MERVIS

CLIMATE POLICY

U.S. Carbon Plan Relies on Uncertain Capture Technology

Talk about unfortunate timing. On one side of the Atlantic Ocean last week, U.S. Environmental Protection Agency (EPA) chief Gina McCarthy was unveiling a landmark proposal to require new coal-fired power plants built in the United States to capture and store at least some of the carbon dioxide they emit. Meanwhile, in Norway, government officials announced that they were scrapping a long-anticipated \$1 billion effort to test carbon capture and storage (CCS) technology on a massive scale at an oil refinery.

The so-called Mongstad project was just the kind of CCS demonstration project that specialists say will be critical to making the technology practical, allowing coal-fired power to satisfy

the proposed U.S. regulations. Its cancellation, after the project went 50% over budget, was part of a discouraging pattern. Over the past decade, "a lot of programs were put in place" to develop CCS, says chemical engineer Howard Herzog of the Massachusetts

Institute of Technology in Cambridge. But "the bad news is they hit a wall." Herzog has documented more than 25 other major CCS projects around the world that have been canceled or put on hold in recent years.

Such setbacks pose a major challenge to President Barack Obama's plans to use CCS to help reduce carbon pollution and curb global warming. If last week's proposal is ultimately adopted, for instance, it would require U.S. utilities building new coal-powered plants to cap carbon emissions at 500 kilograms per megawatt hour—roughly half what an average coal plant emits. CCS could enable a plant to comply, and EPA officials say there are a variety



Fired up. Proposal would require coal-burning power plants to capture some carbon emissions.

CREDIT: DAVID GRAY/REUTERS/NEWS.COM

of existing and nearly ready methods companies could use. But few full-scale CCS installations—which could trap about half a million kilograms of carbon dioxide per year or more—have been built to test those technologies.

Not that governments and the power industry aren't interested. Congress has given the U.S. Department of Energy some \$6 billion to spend on CCS R&D since 2008, with a goal of bringing five large-scale demonstrations online by 2016. That investment has borne some fruit, says Jeffrey Phillips, who manages the CCS research program for the Electric Power Research Institute in Palo Alto, California. It has helped cut by more than 10% the amount of energy that it takes to run state-of-the-art CCS processes, for instance, which is a major issue in the industry. But “is that enough to convince an energy executive to spend \$2 or \$3 billion” on a new coal-fired power plant with CCS? “Absolutely not,” he says.

Skyrocketing prices for commodities, such as steel, have made building new coal-fired plants more expensive than ever—and CCS can add an estimated 30% to the price tag. CCS would also increase operating costs. One pilot project in New Haven, West Virginia, suggested that power produced by a full-scale CCS facility would cost 50% more than from a traditional coal plant. And regulators are reluctant to pass such extra costs on to consumers.

At the same time, the plummeting price of natural gas has made gas-fired plants more attractive, putting even conventional coal plants at a disadvantage. (The proposed rules cover natural gas plants, too, but most are already clean enough to qualify.) Such trends—combined with the rules proposed by the Obama administration—“could mean that [U.S.] power generation companies may completely give up on coal” for new plants for the foreseeable future, Phillips says.

Others are more optimistic. The new proposal—now out for public comment and certain to face legal challenge—also provides incentives to develop CCS, says John Thompson, an analyst with nonprofit Clean Air Task Force in Boston. “Flexible” rules allowing power generators to phase in CCS over time, for instance, could give firms an opportunity to experiment and innovate.

In the short run, “these regulations don't change much,” Herzog says, because no new coal plants are on the drawing board. He says a second Obama proposal, which will restrict emissions from existing coal plants, “could be more consequential.” That is expected next year.

—ELI KINTISCH

AUSTRALIA

Will New Government Overcome ‘Symbolically Challenged’ Start?

SYDNEY, AUSTRALIA—Australian scientists could be excused for feeling uneasy. Fresh off its victory in the 7 September elections, the new conservative government began dismantling climate change science and mitigation programs, and it eliminated the science minister post—leaving the country without a science minister for the first time in more than 80 years. More tremors could follow: The government has not tipped whether it will follow through on a campaign threat to micro-manage Australian Research Council (ARC) grants, or whether it will shore up participation in international science projects.

For the time being, scientific leaders are quietly making their case, hoping that the government views science more favorably than its early moves suggest. “At this stage, it's wait and see,” says Les Field, secretary for science policy at the Australian Academy of Science.

Before the election, the conservative coalition vowed to maintain support for medical research—the government is spending AUD \$780.5 million this year—improve science teacher training, and support industrial R&D. Science will not be shortchanged in the Cabinet, insists Industry Minister Ian Macfarlane, whose duties include oversight of energy, science, and technology research, as well as research infrastructure. Macfarlane tells *Science* that he will work with other ministers to craft a “long-term strategy” for Australian science. And the government will retain its chief scientist, a post now held by neuroscientist Ian Chubb. “My grandfather was chief government geologist in Queensland and my mother worked as a scientist also,” says Macfarlane, who was a farmer before turning his hand to politics. “So I know why science is important in theory, and I've seen it in action.”

Yet climate science is clearly in the crosshairs. On 8 September, Environment Minister Greg Hunt abolished the Climate Commission, a government body established in 2011 to provide independent information on the science of climate change. (Commissioners this week decided to continue operating as a nonprofit. “We've had an outpouring of sup-

port from around the country,” says Commissioner Will Steffen, of the Australian National University.) Hunt also began drafting legislation to eliminate the Climate Change Authority, set up last year to advise government on climate change mitigation.

There is also widespread disquiet in the research community over a campaign pledge by conservative politicians to audit “increasingly ridiculous research grants awarded after expert review by the ARC.” They declared an intention to “reprioritize” AUD \$103 million over 4 years by terminating grants in areas such as anthropology and philosophy and instead funding projects in dementia, bowel cancer, diabetes, and tropical health.

The prospect of “nonexperts” meddling with peer review is “scary,” Field says.

Academy officials met privately with Macfarlane last week, calling his attention to a briefing paper that they had prepared before the election. The confidential document covers the need for a long-term science strategy that isn't “hamstrung” by the electoral cycle, the importance of international collaboration and science literacy, and the difficulty of putting a dollar value on basic research.

Although the new government's rhetoric is alarming, science has been “on the back burner” for years, says Brian Schmidt, an astronomer at the Australian National University in Canberra who has joined colleagues from several organizations to advocate a national research policy. They hope to secure an extension of the National Collaborative Research Infrastructure Strategy, which has poured roughly AUD \$635 million into major projects since 2004 and is set to expire in 2015. Among the projects at risk, Schmidt says, are Australia's site for the Square Kilometre Array radio telescope project and participation in Gemini, which supports telescopes in Hawaii and Chile.

The new government has gotten off to a “symbolically challenging” start, Schmidt says. But he's willing to give it time to come through on substance.

—LEIGH DAYTON

Leigh Dayton is a writer in Sydney, Australia.



Reassuring. Ian Macfarlane says he knows “why science is important.”

INFECTIOUS DISEASES

DNA Sleuths Track *C. difficile* Infection Routes

Studies that track one of the most common hospital-acquired infections suggest that current efforts to control it are missing the mark, by a large margin.

The bacterium *Clostridium difficile* sickens 336,000 Americans each year and kills 14,000, according to figures released last week by the Centers for Disease Control and Prevention (CDC)—a 400% increase in deaths and hospitalizations since 2000. Infection can cause watery or bloody diarrhea so severe that the kidneys fail from dehydration. To control outbreaks, thought to spread mainly through patients' feces, hospitals isolate people with symptoms and require health care workers to wear gloves, frequently wash hands, and decontaminate everything from toilets to windowsills to call buttons with bleach.

But a study this week in *The New England Journal of Medicine* (NEJM) by researchers at the University of Oxford and the University of Leeds, both in the United Kingdom, reports that people with symptoms transmit only about 30% of all hospital infections. That leaves the source of about 70% unaccounted for—and medical experts uncertain about how best to prevent them.

Previous research, including a 2012 study by the same group, had implicated other sources of infection, says Stephan Harbarth, an infection control expert at the Geneva University Hospitals in Switzerland. But the new study nails it, he adds. "It's a huge bit of work and laudable what they have done."

In the NEJM study, the researchers analyzed stool samples collected from approximately 15,000 patients admitted to one of three hospitals near Oxford over 3.6 years, then used whole-genome sequencing to determine which of the infections were linked. Any two cases were considered to be related if they met two conditions: their bacteria differed by less than 2 nucleotides, and the patients had direct or indirect contact while one was infectious or any two overlapped in the hospital during the same 12-week incubation period.

Only 35% of cases could be matched in this way. Furthermore, 13% of positive cases were linked genetically but not epidemiologically—the researchers found no evidence of contact between the patients carrying the related strains, inside or outside of the hospital.

There are three possible explanations, says Mark Wilcox, a medical microbiologist at Leeds Teaching Hospitals. *C. difficile* is known to spread through airborne spores, believed to survive for up to 5 months. If spores survive much longer than that, they might account for many new cases. Another possibility is that *C. difficile* may have yet-to-be-identified reservoirs in the environment or in hospitals. Or the bug might be spreading from the many people who carry

of patients acquired the infection from people with no symptoms, they reported online on 13 August in *Clinical Infectious Diseases*. Work by Prameet Sheth at the University of Toronto and colleagues suggests that more people carry the bug "silently" than previously thought. When they screened patients admitted to general medicine wards, 50 out of 474 tested positive for the bug, although only five had active infection.

If asymptomatic carriers transmit about 30% of infections, that still leaves 30% to 40% for which the source is unknown. "What's the prevalence of asymptomatic carriers?" Harbarth asks. "What's the true rate of transmission in the community? And what the heck can explain the source of these community acquisitions? Is *C. difficile* coming from food, water, soil?"

During a hospital outbreak, targeting the symptomatic may still be the best control strategy, Wilcox says. "If they are spreading the brown stuff around, they will have more case-to-case transmission," he explains. But in hospitals that already have good *C. difficile* control practices, "focusing on symptomatic people may be missing the trick," he says.

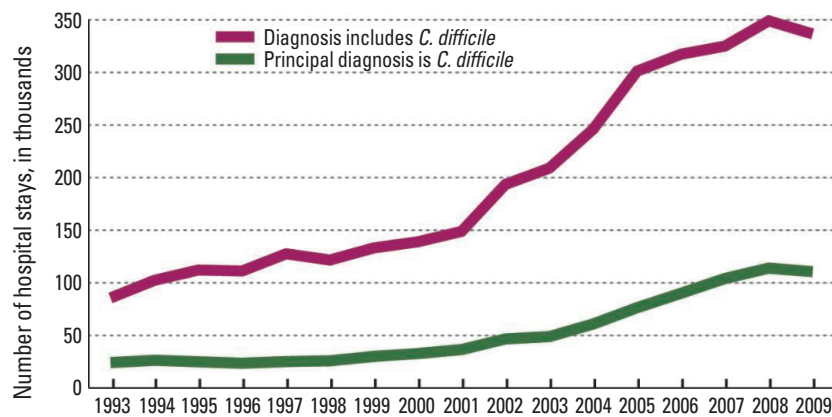
Recent evidence from the United Kingdom suggests that one of the most cost-

effective prevention approaches is to be more prudent about the amount and types of antibiotics prescribed to prevent triggering active cases in the first place. After introducing changes to doctors' antibiotic-prescribing habits, the U.K. government has documented major drops in prescriptions for fluoroquinolones and cephalosporins—broad-spectrum antibiotics—over the past 5 years. Concurrently, since peaking in 2007, *C. difficile* infection rates in the United Kingdom have plummeted more than 70%. Unfortunately, says Cliff McDonald, a medical epidemiologist at CDC in Atlanta, such strategies are still more the exception than the rule in U.S. hospitals.

—GUNJAN SINHA

Gunjan Sinha is a science writer based in Berlin.

Trends in Hospital Stays Associated With *C. difficile* Infection



Alarming trend. Hospital stays for *C. difficile* have climbed sharply. New studies suggest that asymptomatic carriers may be spreading the disease.

C. difficile asymptomatically in their gut. They sometimes become ill when they take antibiotics that destroy gut flora and enable the bacteria to flourish. But even when they are free of symptoms, *C. difficile* carriers shed the bug in stool, but not as much, and likely also spread it when they don't wash their hands.

The prevailing view has been that carriers who are not ill contribute just a few percent to hospital infections. But two other recent studies suggest that such carriers play a much larger role in disease transmission. Scott Curry and colleagues at the University of Pittsburgh School of Medicine screened 3006 patients with multilocus tandem repeat genotyping—similar to DNA fingerprinting—and found that about 30%



Beefing up. Implanted growth promoters help many U.S. cattle gain significantly more weight.

ECOLOGY

Zombie Endocrine Disruptors May Threaten Aquatic Life

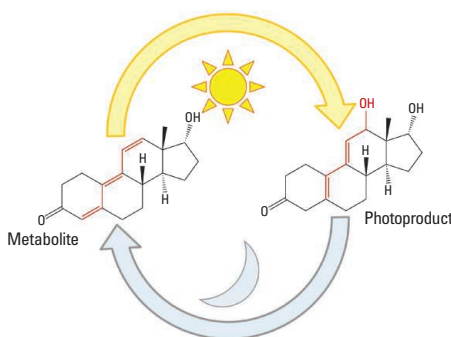
When cattle arrive at feed lots in the United States to be fattened up, many ranchers implant slow-release pellets of steroid hormones to enhance their growth. Only trace amounts of the added hormones remain in the beef after slaughter, but their metabolites can leach from urine and manure. When they reach streams or ponds, some can disrupt the endocrine systems of aquatic life. Industry scientists have noted that many of these metabolites quickly break down in sunlight, which should limit the ecological threat. But they did not reckon with what happens next.

Research online in *Science* shows that the degradation products of a widely used synthetic androgen called trenbolone acetate can revert at night, zombielike, back into the endocrine-disrupting metabolites (<http://scim.ag/ShenQu>). The same could be true of metabolites of other hormones that make their way into the environment. “This is a very comprehensive and impressive study, and its implications for aquatic ecosystems are sobering,” says Karen Kidd of the University of New Brunswick, Saint John, in Canada. “It’s a potential game-changer” for ecological risk assessment, adds Deborah Swackhamer of the University of Minnesota, Twin Cities. “My eyebrows went up.”

Citing human health concerns, Europe banned trenbolone and other bovine growth-promoters in 1981. Yet the U.S. cattle industry enjoys an estimated \$1 billion annual benefit from trenbolone alone: Animals receiving it gain up to 20% more weight per day than they would otherwise and are also more efficient at turning feed into muscle. But environmental concentrations as low as 10 parts per trillion significantly reduce the fecundity of fish and skew the sex ratios of their populations,

scientists from the U.S. Environmental Protection Agency have found.

In 2009, environmental engineer David Cwiertny of the University of Iowa in Iowa City and Edward Kolodziej of the University of Nevada, Reno, set out to trace the fate of the hormone and its metabolites. In their early work, Cwiertny and Kolodziej confirmed that the metabolites quickly degrade in sunlight as photons transform a part of their structure called a π -bond. But they also found that the so-called phototransformation products retain some ability to alter the endo-



Cyclic. Sunlight turns trenbolone metabolites (left) to photoproducts but changes (red) revert at night.

crine function of laboratory fish, as their team detailed in the 21 May issue of *Environmental Science & Technology*. “The risk isn’t necessarily going to go away,” Cwiertny says.

Cwiertny’s graduate student Shen Qu then discovered that, at night, a fraction of the two major photoproducts reformed into their parent molecule, the trenbolone metabolites. “That alone is an astonishing result,” Swackhamer says. The authors also observed this unprecedented reversible phototransformation of steroids in dienogest, which is used

for human birth control, and in the illegal bodybuilding drug dienedione.

What happens, Cwiertny and Kolodziej believe, is that sunlight adds a water molecule across the π -bonds, creating the photoproduct. At night, a chemical reaction spontaneously removes the water. This happens at temperatures and pH typical of surface waters known to be contaminated by trenbolone metabolites. “It makes total sense when you look at it,” says Douglas Latch, a photochemist at Seattle University in Washington. “It’s unexpected in the sense that no one had designed experiments to find that.”

The team found that only about 15% of the metabolites is reformed from the photoproducts during 12 hours of darkness. But the prolonged darkness at the bottom of murky ponds or in the pore-water of streambed sediment may allow much more reversion. After 120 hours without light, the researchers found, up to 70% may be reconverted. For added realism, the team studied trenbolone metabolites added to water taken from the Iowa River, as well as in pond water with manure from implanted cattle. The trenbolone metabolites in the contaminated water underwent the same diurnal cycles of reversion seen in laboratory samples, Cwiertny says.

Regulators need guidance on how to incorporate these findings into risk assessment, says Kathrin Fenner of the Swiss Federal Institute of Aquatic Science and Technology in Dübendorf. “They cannot just be ignored.” The researchers themselves say that the implications for human health are minimal, given the small amounts of trenbolone in meat, but that the resurrected endocrine disruptors could be harming aquatic life. Cwiertny adds that the reversion process yields not just the original metabolites but also creates similar chemicals called structural analogs that might have unknown effects. “You’re opening the door to additive toxicity,” he says.

Ecotoxicologist John Sumpter of Brunel University in Uxbridge, U.K., points out that the new study does not evaluate the biological effects of any of these compounds, so it’s impossible to judge whether the photoreversion has any significant ecological impact. A spokesperson for Merck, a large producer of trenbolone, agrees, saying that “the implications of these findings to the environment remain undefined and theoretical.” Nonetheless, Sumpter cautions: “Just because a chemical is degraded, don’t assume it won’t come back to haunt you.” —ERIK STOKSTAD

CREDITS (TOP TO BOTTOM): DAVID R. FRAZIER/DANIEL MONT; DAVID M. CWIERTNY/UNIVERSITY OF IOWA



Taming a Mercurial Element

AT ROOM TEMPERATURE, MERCURY CAN PUDDLE IN YOUR PALM like some silver elixir. Ancient alchemists believed the glittering metal was the origin of all matter; healers saw it as the key to eternal life.

In fact, however, mercury is a potent toxin that causes cell death, brain damage, and birth defects. Unfortunately, it is also a widespread byproduct of common industrial processes, such as burning coal, and is widely used in trades such as gold mining. Humans have released some 200,000 metric tons of mercury into the environment since 1850, researchers estimate, with sometimes staggering costs to human health.

On 9 October, representatives from more than 140 nations will

gather in Japan to officially embrace a global agreement aimed at reducing mercury's threat—and spurring the research needed to do so (see story, p. 1443). Fittingly, the delegates are gathering in Minamata, a seaside town that was devastated by mercury pollution a half-century ago and is still struggling with that legacy (see story, p. 1446). The new convention seeks to prevent similar disasters elsewhere, such as in the world's rapidly expanding artisanal gold mines, which are now the planet's leading source of new mercury pollution (see story, p. 1448).

Mercury's, well, mercurial properties make it hard to get a grip on. But the world is now going to try.

—DAVID MALAKOFF

CREDIT: SEAN HAWKEY/WWW.HAWKEY.CO.UK

With Pact's Completion, The Real Work Begins

The Minamata Convention on Mercury seeks to curb or end most uses of mercury. It also calls for plenty of research

STOCKHOLM—An elegant crematorium sits in a wooded cemetery here on the edge of the city. It once reduced corpses to ash. But government regulators shut it down not long ago, because its old-fashioned ovens couldn't prevent toxic mercury in the dental fillings of the dead from wafting into the atmosphere, and ultimately falling back to Earth and threatening the living.

Thousands of similar crematoria, and countless other industrial sources of mercury, could eventually face similar regulation as a result of the Minamata Convention on Mercury to be finalized in Japan next month. Years in the making, the agreement calls on signing nations to launch a wide range of initiatives to reduce mercury pollution, which can cause birth defects, brain damage, and disease in humans and other animals. Goals include curbing mercury emissions from coal-fired power plants and industrial facilities, phasing out by 2020 many consumer products that contain mercury, "phasing down" the use of mercury in dental amalgams, and closing all mercury mines within 15 years after the convention takes effect. (Use of existing mercury in research and medicine, however, could continue.)

It's an ambitious to-do list, and many of the convention's goals are essentially voluntary, qualified with the phrase "where feasible." But modeling studies suggest that it could, under aggressive regulatory scenarios, help reduce global mercury emissions by 15% to 35% in coming decades.

Beautiful killer. Mercury, here on a gold miner's thumb, can cause brain damage and birth defects.

Still, even some who welcome the convention worry it doesn't go far enough, fast enough. Negotiators stopped short, for instance, of requiring many industrial and

Online sciencemag.org

Podcast interview with author David Malakoff (http://scim.ag/pod_6153).

power plants to rapidly curb their mercury emissions, giving some a decade or more to act. "That's a lot of mercury" that will be added to the environment in the meantime, says Susan Egan Keane, an environmental analyst at the Natural Resources Defense Council in Washington, D.C. But "the reality is that we wouldn't have had a treaty at all if we didn't have those compromises."

of protecting human health and developing science-based methods of monitoring people at risk of exposure. Such data will be essential to determining whether the convention is achieving its goals—and to enforcing any new mercury controls.

"Under no circumstances do I think this treaty is a silver bullet," says Henrik Selin, a policy researcher at Boston University. But it will become "a focal point" for science and innovation, he predicts.

Trust but verify?

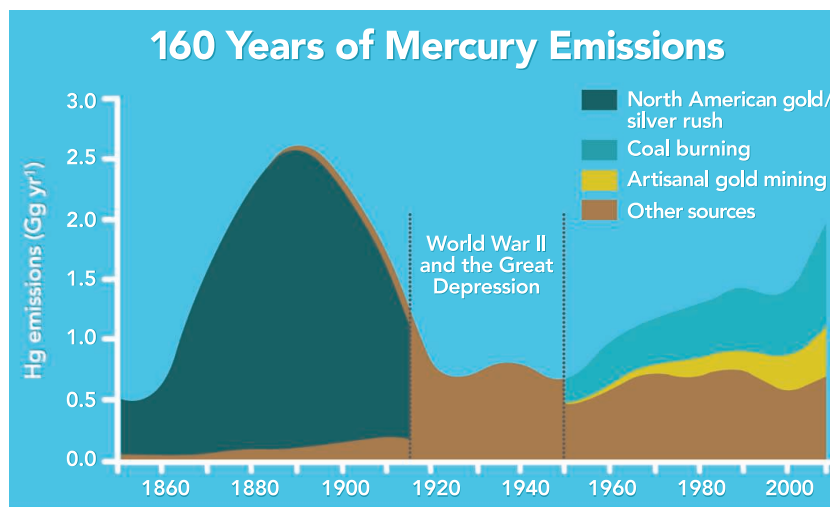
One major task will be clarifying how mercury enters and moves through the environment (see top graphic, p. 1445). Today, researchers estimate that human actions annually dump some 2000 metric tons of the heavy metal into air and water. That's down from an estimated peak of some 2600 metric tons per year in 1890, during a gold and silver mining boom in North America (see graph, below).

But there's plenty of uncertainty in those numbers: The United Nations Environment Programme (UNEP), for instance, recently

estimated that annual air emissions could total as little as 1010 metric tons or as much as 4070. Even less certain are the amounts released into water, and the quantities "re-released" into the environment by the vast stores of mercury that have piled up in soils and sediments over the past few centuries. Overall, studies suggest that these "re-emissions" account for about 60% of annual air emissions, double the share contributed by current sources. (Natural sources such as volcanoes and forest fires

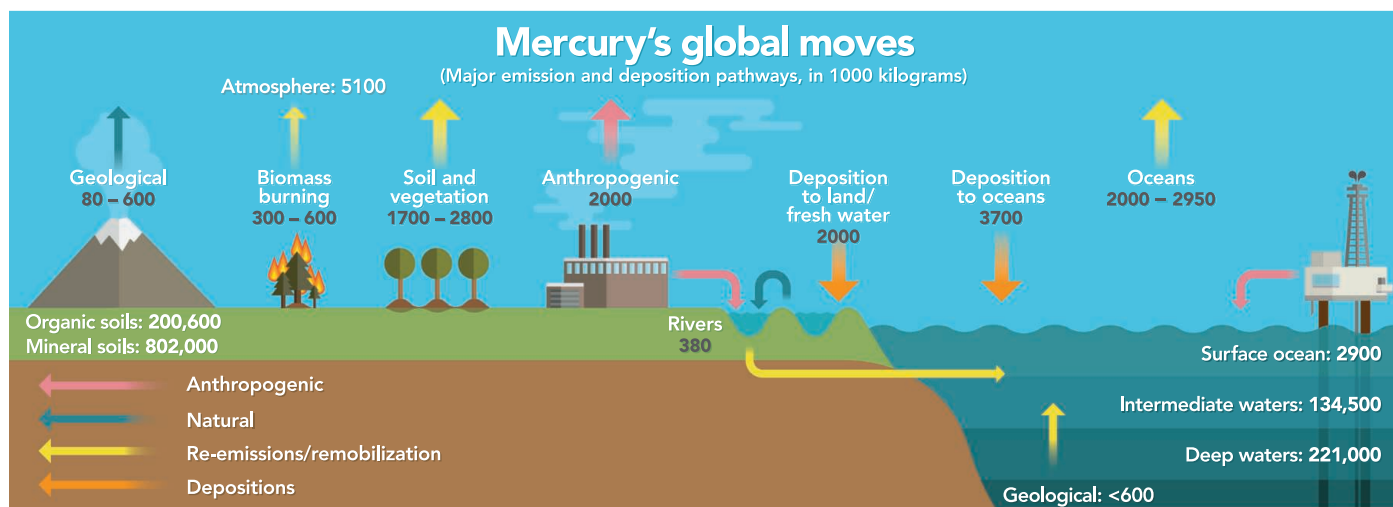
account for 10%.)

Firming up such figures is a key goal of the new convention, which calls on signers to submit detailed—and accurate—emissions reports. Some existing data gaps might be filled simply by improving tracking of mercury purchases or requiring industries to file more detailed pollution reports. But assembling truly solid



Peaks and valleys. Studies suggest mercury emissions surged before and after the chaos of World War II and the Great Depression.

The pact also includes plenty of work for researchers. It tasks nations with beefing up efforts to monitor and tally mercury releases from a slew of sources (see bottom graphic, p. 1445). It also calls for improving efforts to understand mercury's complex chemistry, and how the toxin moves through food chains and ecosystems. And it includes landmark language emphasizing the importance



data could also require upgrading the world's existing patchwork of permanent monitoring stations, which feature sensitive instruments able to detect specific mercury compounds. "We have to be able to verify [a country's] emissions inventory ... by cross-checking with monitoring and real data," says Nicola Pirrone, director of the Institute of Atmospheric Pollution Research in Rome.

Creating an effective global monitoring system could be a tall task. Although the United States, Canada, and Europe have run relatively robust mercury measurement programs for more than a decade, other regions, especially in the Southern Hemisphere, are barely covered. In one nascent effort to change that, in 2010 Pirrone won support from the European Union to launch a Global Mercury Observation System. In cooperation with others, it has begun installing instruments in places such as Brazil and Argentina. But its future is unclear; the European Union promised just 5 years of funding, and the new instruments are expensive, costing tens of thousands of dollars to build and operate. And although the Minamata Convention calls on wealthier nations to help poorer ones boost their data-gathering capabilities, so far there is no concrete funding plan.

Studies have also suggested that some commonly used instruments may not be able to detect important varieties of mercury that form in the atmosphere, giving a misleading picture of emissions and their final resting place. Such

findings are a reminder that much of mercury chemistry is still a mystery; scientists don't understand key oxidation processes in the atmosphere, for instance, or how the metal moves and behaves at high altitudes. "If we don't know [such atmospheric chemistry], we don't know anything," says Dan Jaffe, an atmospheric chemist at the University of Washington, Seattle.

Healthy accord

Although the agreement doesn't spell out exactly how its human health provisions should be implemented—or how the work would be paid for—past agreements could

Making tracks. Mercury emissions can move vast distances around the planet in air and water, settling for centuries before being re-emitted to the environment in new forms.

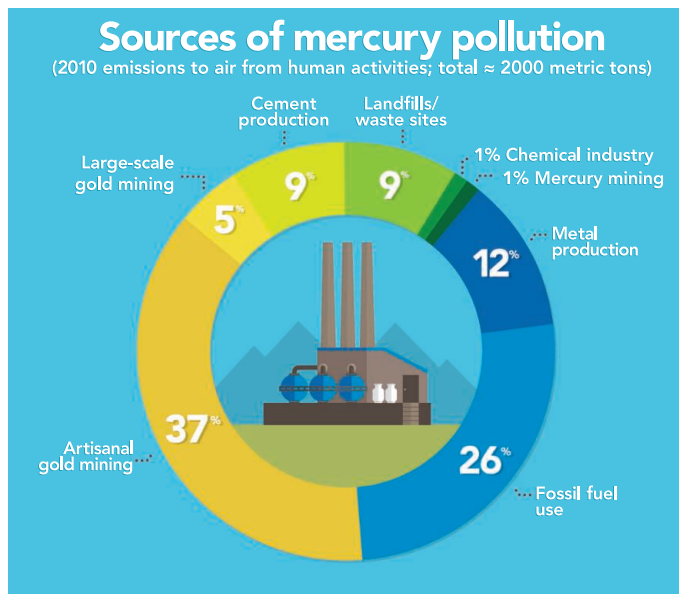
record levels of pesticides and other chemicals in breast milk in order to track infant exposures. Signers of the Minamata Convention could choose to pursue that kind of biomonitoring and reporting infrastructure for mercury, too.

Countries still need to work out what kinds of data to collect, however. Although blood samples can provide extensive information on total mercury exposure, for instance, they can be intrusive to collect and costly to maintain. An alternative might be gathering hair or urine samples, but recent research has shown that each might accumulate a different record of mercury exposure, potentially skewing results. There is also the question of which populations should get priority for biomonitoring—and how to make sure they derive some benefit from research.

Such issues are likely to come to the fore as nations begin to implement the Minamata Convention, which will enter into force once 50 nations have ratified it. Then, the clock starts ticking: Nations are supposed to meet for an initial "effectiveness evaluation" within 6 years. By then, researchers hope to be

able to report on whether efforts to limit mercury pollution are on track, or are still falling short.

—NAOMI LUBICK AND DAVID MALAKOFF



Shifting shares. Artisanal gold mining has become the world's leading source of mercury emissions in recent years.

provide a guide. Under the 2001 Stockholm Convention on Persistent Organic Pollutants, for instance, nations ultimately agreed to create coordinated databases that



MERCURY POLLUTION

In Minamata, Mercury Still Divides

Nearly 60 years after a chemical plant caused one of the world's worst episodes of mercury poisoning, the Japanese town that came to symbolize the metal's threat is still struggling with the aftermath

MINAMATA, JAPAN—Anyone arriving here by train gets the message that this is a company town: The station doors open to a broad tree-lined street that leads directly to the gates of what was once the Shin Nippon Chisso Hiryo chemical plant. The street bisects downtown, mirroring a deeper division in the community that has given its name to the Minamata Convention on Mercury.

Nearly 6 decades after researchers identified the first victims of what came to be called Minamata disease, and 45 years after the government officially recognized that methylmercury discharged from the Chisso plant had caused one of the most notorious pollution disasters of all time, this community and scientists are still split over the calamity, and in some very curious ways. The town hosts both official and “unofficial” Minamata disease museums, for instance. There are also twin disease research institutes, one sponsored by the government, the other by a university.

The two blocks are not—on the surface—antagonistic. “We may have different perspectives, but that doesn’t mean we are enemies,” says Kunio Endo, a director of the Minamata Disease Center, a citizens’ group which operates the unofficial Minamata Disease Museum. But the differences in style and emphasis are clear. The unofficial

museum is housed in a tin-roofed shed that sits along a narrow lane high above the town. On a hot, humid day, breezes through open doors provide only occasional relief.

In contrast, the official Minamata Disease Municipal Museum overlooks the bay in a sleek, modern building that is comfortably air-conditioned. And while the municipal museum’s slick displays tend to reflect the official line that the Minamata incident is largely resolved, its unofficial counterpart stresses patients’ and advocates’ view that the tragedy is still unfolding. “There are still many unresolved issues,” says Masanori Hanada, a social policy specialist who heads the Open Research Center for Minamata Studies, attached to Kumamoto Gakuen University.

Among them: finding ways to identify patients with less severe mercury poisoning symptoms, and figuring out how low-level or early-life exposure is affecting people as they age. There are also fierce, ongoing battles over who should be counted among the victims of Minamata disease, who could number just a few thousand or up to 100,000—depending on who is counting.

A historic divide

The split in the Minamata community existed even before the disease emerged. The company that ultimately became Chisso

Toxic legacy. Decades after mercury pollution ended in the town of Minamata, victims still need help.

Corp. opened a fertilizer plant in the town in 1908 and gradually diversified into a range of chemicals. In 1932, Chisso began making acetaldehyde, used to produce a range of synthetic materials. The production process relied on mercury sulfate as a catalyst, producing an unwanted byproduct known as methylmercury—a potent form of mercury now known to enter the food chain and accumulate in fish and other animals and cause brain damage and birth defects.

As Chisso prospered, Minamata grew, and its economy bifurcated. By the 1950s, roughly one-half of the town’s residents depended directly or indirectly on the company for their livelihood. The other half stuck with their traditional livelihoods: fishing and farming. In the spring and summer of 1956, what was first known simply as “a strange disease” started afflicting fishing families in villages scattered around Minamata Bay. Symptoms included numb hands and feet and difficulty walking and talking. Severe cases progressed to convulsions and death. Of 54 patients identified in 1956, 17 died that year, according to the National Institute for Minamata Disease, the “establishment” research institute.

By year’s end, Kumamoto University researchers believed the cause was eating local fish and shellfish contaminated with heavy metals. Suspicion focused on the Chisso plant, which discharged its untreated waste directly into waterways. But for several years, Chisso refused to disclose any information; investigators later found that executives covered up in-house research suggesting a link between its wastewater and the illness, and put forward alternative theories.

Residents were divided, either blaming or defending the company. Victims and their families were ostracized; at first, many townspeople feared the illness might be contagious.

In 1959, under pressure from the fishing industry and the national government, Chisso installed a wastewater treatment system. It was of little use, according to a 2011 report prepared by Japan’s Ministry of the Environment. “It was later discovered,” the report noted, “that the system was not designed to remove mercury and was useless for the removal of methylmercury.” In December 1959, Chisso agreed to compensate known victims and fishers, although the company continued to push alternative theories for what was causing the illness, by then dubbed Minamata disease.

For the next 9 years, Chisso and national and local governments considered the case closed. Officials were concerned about the economic impacts of closing or reengineering the plant. So the wastewater taps remained open, and methylmercury continued to accumulate in the tissues of fish and shellfish. The metal also continued concentrating in those who ate seafood, including in the placentas of expectant mothers, exposing the unborn babies to a potent neurotoxin.

Finally, in May 1968, Chisso halted acetaldehyde production, thus ending its use of mercury. (The plant still makes other products.) Later that year, the national government officially acknowledged that Minamata disease was caused by eating seafood contaminated by effluent from the factory. But the statements claimed the outbreak had ended in 1960. And through the early 1970s, the national government continued to play down the threat of eating contaminated seafood from Minamata Bay, ignoring the persistence of mercury in seafloor sediment. Government officials didn't close the bay to fishing until 1975 (lifting the ban in 1997 after massive dredging). Meanwhile, activists started drawing global attention to the growing human toll with dramatic photographs of disfigured, paralyzed victims. "If the national government had taken any measures to stop people from eating fish, we would have had many fewer patients," Endo says.

Who is a patient?

Exactly who those patients are is the subject of much medical and legal dispute, fed by uncertainty about exposure levels and symptoms. The year Chisso stopped using mercury, Minamata patients and advocates formed the Citizens' Council for Minamata Disease Countermeasures to support victims seeking compensation. Since then, there have been 45 years of negotiations, arbitrations, court judgments, bureaucratic diktats, and Cabinet decisions, as well as two laws enacted by the national legislature. The result: a bewildering patchwork of differing classes of victims, levels of compensation, and medical support.

There are, for instance, some 2260 "Certified Minamata Disease Patients" selected by expert panels using strict criteria set by the environment agency in 1977. To qualify, a person must have more than one "severe" symptom, have lived in the most contaminated areas, and been born before 1969. Each has received a lump-sum payment ranging from \$160,000 to \$180,000 plus pensions and medical care.

Under a 1995 national law, another 11,000 patients with less severe abnormalities

became eligible for payments of \$26,000 and support for medical expenses. And in 2009, the national legislature created yet another program to support those with even less severe disabilities.

Last April, however, two Supreme Court decisions threw the system into turmoil, overruling one of the expert panels that certifies victims and questioning whether it was scientifically justifiable to require patients to exhibit multiple severe symptoms to qualify. Meanwhile, 65,000 people have applied for compensation under the 2009 law.

Endo says he believes the total number of

Rehabilitation Clinic in Minamata, has been doing its own surveys. At the International Conference on Mercury as a Global Pollutant in Edinburgh in July, they reported finding extensive signs of methylmercury poisoning in more than 1000 people living on the coast of the Shiranui Sea, which contains Minamata Bay. "These people are not like the victims seen in the [famous] photographs, but they are Minamata disease patients," Takaoka says.

The long view

More patients could emerge as Minamata's population ages, Takaoka says. His surveys



Ground zero. Minamata disease was ultimately linked to a chemical plant in the community.

people afflicted with Minamata disease could be as high as 100,000. One part of the challenge, critics of the current system say, is that screening for signs of methylmercury poisoning was done several years after the crisis and in a piecemeal way. Another is that even people who were exposed to biologically harmful levels may not be aware they have symptoms. "I cannot distinguish by feel whether I'm touching someone's hair or their shirt," says Hajime Sugimoto, a 52-year-old, third-generation Minamata fisherman who lived in Tokyo and worked odd jobs during the ban on fishing. He says he also cannot tell when he has his hands in cold water. But when he was a child, "I thought this was just normal."

To help identify other such "quiet" victims, a team led by Shigeru Takaoka, a physician at the private Kyoritsu Neurology and

suggest that symptoms may appear or worsen as those exposed get older, but so far there are no large-scale, systematic studies.

The slow, late, and cumbersome process of identifying Minamata patients is somewhat ironic, critics note, given that the new convention includes unusual language that commits signers to efforts to understand and monitor the health threats posed by mercury (see story, p. 1443). But it may be in keeping with the conflicted views that many Minamata residents have of the horrendous event that gave the town global recognition. Although the Japanese government successfully lobbied hard to get the convention named for Minamata, Endo says that 90% of residents "are not happy about an incurable disease being named after their hometown."

—DENNIS NORMILE



MERCURY POLLUTION

Gold's Dark Side

Small-scale artisanal gold mining has become the world's leading source of mercury pollution, poisoning air, rivers, and people

People come to La Rinconada, Peru, for one major reason: gold. There's little comfort, and not much oxygen, to be had in the world's highest city, which climbs windswept slopes at an altitude of more than 5000 meters. But the surrounding Andes mountains are full of the precious metal, and more than 35,000 people have flocked there to mine it, trade it, or otherwise strike it rich.

But if there's gold in the hills, there's a far more sinister element lurking in the city's air, water, and food: mercury.

Endowed with a unique ability to extract gold from low-grade ore, mercury remains the method of choice for artisanal gold miners working near La Rinconada and elsewhere in Latin America, Asia, and Africa. Often very poor, these miners work alone or in small groups, using mercury to separate and bind flecks of gold from soupy slurries of water and sediment. They are outlaws in many countries, eking out a living on the margins of the formal economy. And yet this diffuse industry is now the world's largest mercury polluter, pumping more mercury into the environment than all the world's coal-fired power plants combined. The mining operations typically leave a trail of mercury waste, putting as many as 100 million people at risk of poisoning.

"If you're going to do something about mercury pollution, you have to do something about small-scale mining," says Susan

Egan Keane, a senior environmental analyst at the Natural Resources Defense Council (NRDC) in Washington, D.C. She helped governments craft language in the Minamata Convention on Mercury that commits signers to reducing or eliminating mercury use in artisanal mining.



High risk. An artisanal miner in Peru mixes mercury into a barrel of ore and water.

Achieving that goal will not be easy, with gold prices soaring and as many as 15 million miners using an estimated 1600 metric tons of the metal in 2012 alone. Weaning them off mercury will require a blend of economic incentives and practical new technologies,

Exposed. Artisanal miners use mercury to trap flecks of gold, then burn off the toxic metal.

and it could be years before a majority of miners are convinced to adopt them.

It is "an extremely daunting problem," says Luis Fernandez, an ecologist and expert in artisanal gold mining at the Carnegie Institution for Science in Stanford, California. And the stakes are high: "Once areas are contaminated, they are contaminated for a long time."

Why mercury?

Add a palmful of mercury to a slurry of ore and "it serves almost like a sponge," Fernandez says. It soaks up gold that's too diffuse to pan, forming a heavy ball of puttylike amalgam that's easy to pluck out (see graphic, p. 1449). Miners and traders then burn off the mercury, often in an open flame, leaving just the gold.

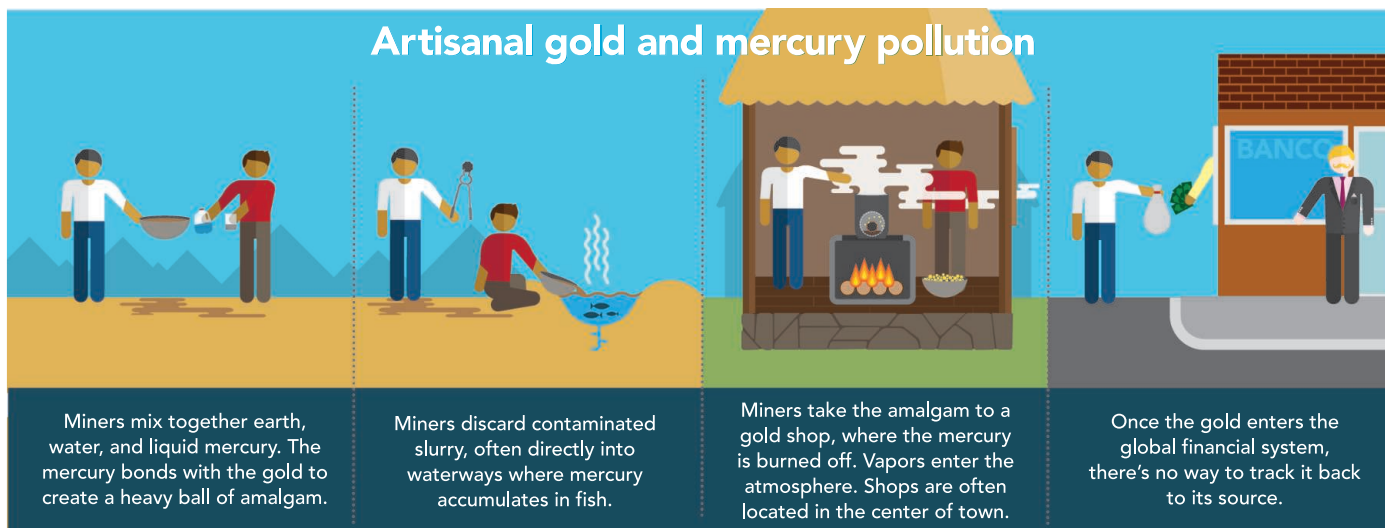
The process creates ample opportunity for pollution—and exposure. Miners simply dump the mercury-infused slurry, which contaminates rivers. Bacteria in the sediments help transform the inorganic mercury to organic methylmercury, which can be absorbed by phytoplankton and accumulate in fish and other creatures higher on the food chain. People who eat contaminated fish are at risk of neurological damage, autoimmune disease, and devastating birth defects. In a new study, Fernandez found that people in the Peruvian state of Madre de Dios, home to much of the country's artisanal gold mining, have mercury levels in hair averaging 3 parts per million, triple the maximum limit recommended by the U.S. Environmental Protection Agency. "For the most part, these are people who are not miners. These are people who eat fish," Fernandez says. Levels are even higher—more than 5 ppm—in the state's indigenous communities, which rely on local fish for protein.

In mining towns like La Rinconada, the threat is more direct: People are exposed every time they take a breath. As gold shops in the center of town burn the mercury-gold amalgams, mercury vapor wafts into crowded neighborhoods. "In effect, some of these little towns have the equivalent of four, five, 10, 20, coal-fired power plants in the center of them," Fernandez says. Meanwhile, mercury lifted into the upper atmosphere can travel vast distances before falling back to Earth and making its way into the global food web.

Data dearth

Documenting exactly how much mercury miners are using—and exactly how it is affecting people—has been a challenge. Artisanal

Artisanal gold and mercury pollution



gold mining often takes place in remote areas, and miners can be wary of scientists, whose findings could threaten their livelihoods. "We've been chased out of towns when we've tried to do surveys or give a talk," Fernandez says. Still, researchers are making progress. The United Nations more than doubled its estimate of mercury emissions from artisanal mining between 2008 and 2013, "mostly due to better reporting," says Kevin Telmer, executive director of the Artisanal Gold Council in Victoria, Canada, which works to reduce mercury use in small-scale mining.

The United Nations may not have the whole picture, however. "The more you look, the more you find," says NRDC's Keane. A 2011 study of the air quality in and around La Rinconada's some 250 gold shops, for instance, concluded that they could be emitting 20 metric tons of mercury per year. That's nearly one-third of Peru's reported annual emissions, suggesting that the official tally is a severe underestimate.

Information on the health effects of artisanal mining is also scarce, because of the challenge of doing long-term studies in poor, remote, and sometimes hostile communities. When Daniel Peplow, an environmental toxicologist at the University of Washington, Seattle, was working with at-risk indigenous communities in Suriname, he recalls that community leaders said: "We're really tired of you people, you scientists, coming here, studying us, taking away information, and not coming back. We're not benefiting from your research." In response, Peplow founded the Suriname Indigenous Health Fund, which helps indigenous communities direct their own research on mercury's impact. Often, he says, they request screening programs to determine who has been exposed, as well as long-term monitoring

to reveal whether efforts to reduce mercury exposure are improving health.

Many observers, however, say the world already knows enough to take action. "There's ample information already available that shows that the people in small-scale mining communities are exposed to mercury, which is a well-characterized neurotoxin," Keane argues. The real issue is finding workable ways of getting mercury out of the global gold trade.

Simply banning artisanal mining—or mercury— isn't a realistic option, specialists have concluded. "Making [artisanal mining] illegal hasn't worked," Keane says. It only demonizes miners and drives their activity further underground, cutting off the very resources they need to improve their practices: education, training, and credit. "You cannot deal with someone who officially doesn't exist," says Jacopo Seccatore, a doctoral student in mining engineering at the University of São Paulo in Brazil.

The only winning strategy, Seccatore says, is creating economic incentives to reduce mercury use. "It's all a matter of efficiency. If [miners] get more gold from their ore than they do right now, they will shift immediately to mercury-free technologies." Indeed, mercury amalgamation often "wastes" more gold than alternative methods, some of which are as simple as running slurry through a box lined with a hairy piece of gold-trapping carpet.

The problem, Seccatore says, is that switching to new techniques requires upfront resources that most miners don't have. "They don't have the structure, they don't have the knowledge, and they don't have the money to give up mercury for some safer technology." Plugging those gaps will require transforming artisanal mining into a more formalized

industry, argues Marcello Veiga, Seccatore's mentor and a mining engineer at the University of British Columbia in Vancouver, Canada. To that end, Veiga and Seccatore are working with the Ecuadorian government to establish the International Training Centre for Artisanal Miners (ITCAM) in Portovelo. By training miners to use environmentally sound techniques and attract investors with business plans, they hope to "turn every artisanal miner into a responsible, small-scale miner," Seccatore says.

Such economic strategies ring true with the gold council's Telmer. "Health [and] environmental messages will fall on deaf ears and have for 30 years," he says. And Latin America is an attractive place to try experiments like ITCAM, he adds, because many of the region's miners are already organized into cooperatives, unlike in Africa and parts of Asia, where miners usually work alone.

The Minamata Convention could also help create economic incentives for change. It calls for ending the primary mining of mercury within 15 years once 50 nations have ratified it, ultimately limiting supplies and likely raising prices. "Inevitably there will be a squeeze on mercury," Telmer predicts. That could help make alternative techniques more attractive to miners, and even encourage gold shops to capture, recycle, and resell mercury they now burn.

Still, specialists fear that the convention may be too little, too late to erase gold's toxic legacy. Ultimately, the agreement may "do a great job stemming the flow of mercury" from artisanal mining and other sources, Peplow says. But the vast quantities of mercury already dumped by artisanal mining will persist in ecosystems for hundreds of years, he notes, and "there's no going back."

—LIZZIE WADE

LETTERS

edited by Jennifer Sills

L'Aquila's Aftershocks Shake Scientists

I HAVE BEEN SENTENCED TO 6 YEARS OF IMPRISONMENT FOR FAILING TO GIVE ADEQUATE advance warning to the population of L'Aquila, a city in the Abruzzo region of Italy, about the risk of the 6 April 2009 earthquake that led to 309 deaths. I have been found guilty despite illogical charges and accusations that set dangerous precedents for the future of the scientific process.

The judge's ruling claims that citizens of L'Aquila would normally rush outside upon feeling an earth tremor, but that they did not in 2009 because a Major Risks Commission (CGR) meeting in L'Aquila, one week beforehand, had given them a false sense of security. However, this meeting was run, not by the National Institute of Geophysics and Volcanology (INGV), but by an arm of the Prime Minister's office: the Civil Protection Agency (CPA). An agreement between the INGV and the CPA states that the latter is exclusively responsible for communicating any state of risk. The INGV has always scrupulously adhered to that regulation. As a former president of the INGV, I never spoke to the media about the seismic situation at L'Aquila, and no relative of the victims suggested otherwise.

Rather, the "proof" used by the public prosecutor was the CGR meeting minutes. At that meeting, I (and others) stated that Abruzzo, and particularly L'Aquila, is one of the worst earthquake zones in Italy. I then explained that earthquakes are not predictable for good scientific reasons and discussed some of the seismic mechanics involved. The Mayor of L'Aquila, Massimo Cialente, testified that he was struck by my statement about the local seismic risk at this meeting, and as a result he decided to close certain schools and recommend a state of emergency be declared.

At the CGR meeting, we also distributed the Seismic Hazard Map, made official in 2003 (1), which was largely the work of the INGV under my presidency. The map clearly shows Abruzzo as a hazardous zone. One of its goals was to inform administrators of what action to take to reduce seismic risk in the areas they governed. The maps were later

made available to the public prosecutor, who ignored them just as he ignored the testimony of Mayor Cialente.

As further evidence of my guilt, the public prosecutor completely distorted the argument of one of my journal publications (2), effectively putting science itself on trial. In that 1995 work, my colleagues and I highlighted the statistical importance of temporal "clustering": various strong earthquakes in a (geologically) brief time span. We posited that the high probability rate calculated for the Aquilan territory is not statistically meaningful because it is based on three events that occurred between the 17th and 18th centuries—hardly a sufficient basis to describe what would happen in subsequent centuries.

The public prosecutor's superficial interpretation of scientific results to bolster his argument sets a grave precedent for not only seismology but many other disciplines as well. Science is constantly evolving; research proceeds by trial and—as knowledge grows—error. When I wrote the "indicted" work, I was addressing my worldwide peers and awaiting their verification, as must be the way of all modern scientific research.

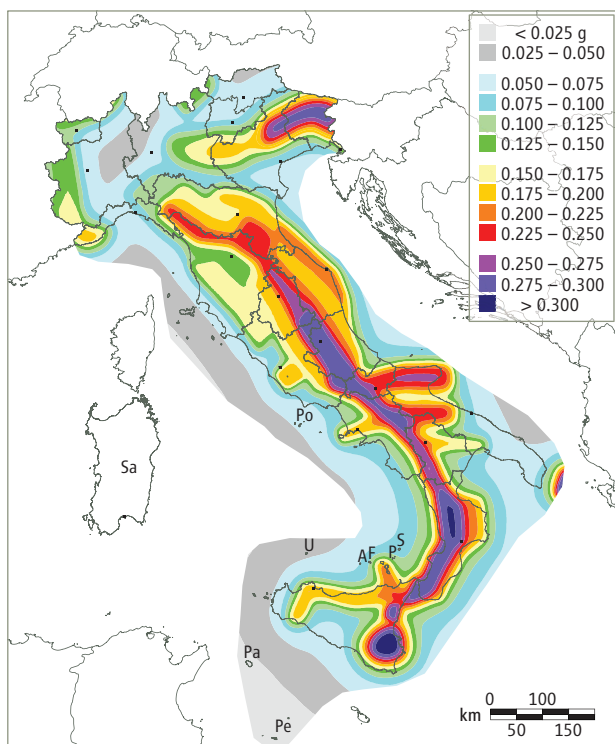
In publishing an official map, seismologists have done all they currently can to protect society from earthquakes. I can hardly be blamed for the poor quality of buildings or for people's failure to conform to anti-seismic laws—these are the responsibilities of other authorities. The local CPA is responsible for accurate communication of risk and effective management of emergency situations. I did not disseminate false or imprudent information. My question is: What could I do to avoid conviction? I suppose I should have foreseen the earthquake!

ENZO BOSCHI

University of Bologna, Bologna, 40126, Italy. E-mail: enzo.boschi@unibo.it

References and Notes

1. M. Stucchi et al., *Bull. Seismol. Soc. Am.* **101**, 1885 (2011).
2. E. Boschi, P. Gasperini, F. Mulargia, *Bull. Seismol. Soc. Am.* **85**, 1475 (1995).
3. I want to express my strong friendship to Giulio Selvaggi, who was also wrongly accused in the L'Aquila trial.



Seismic Hazard Map distributed by the National Institute of Geophysics and Volcanology.

CREDIT: INGV

Low Marks for Education Funding Priorities

ANYONE INVOLVED SUBSTANTIVELY IN SCIENCE education during the past five decades will see the irony in the decision by the Office and Management and Budget (OMB) to trim the federal government's science, technology, engineering, and mathematics (STEM) programs on the grounds that many of them lack evaluation data on efficacy ("An invisible hand behind plan to realign U.S. science education," J. Mervis, *News Focus*, 26 July, p. 338). Although federal funding often supported formative evaluation (assessment in the pilot phase to improve the program itself) during the development of new curricula, it was virtually impossible to secure funding for summative evaluation (assessment of effectiveness after implementation) because of the costs and time frames involved. At the Biological Sciences Curriculum Study (1), where the value of summative evaluation always has been self-evident, we often lamented that the federal government funded a series of 90-meter dashes, supporting development of new instructional materials but not their evaluation. Funding from the Institute for Education Sciences for efficacy trials (2) that provide one type of summative evaluation constitutes some progress, but it is not enough.

It is perverse for OMB to blame STEM projects for deficiencies that were inherent in the government's funding priorities. Perhaps an evaluation of those priorities is in order.

JOSEPH D. MCINERNEY

Executive Vice President, American Society of Human Genetics, Bethesda, MD 20814, USA. E-mail: jmcinerney@ashg.org

References

1. Biological Sciences Curriculum Study (www.bsos.org).
2. J. K. Spybrook, S. W. Raudenbush, *Educ. Eval. Pol. Anal.* **31**, 298 (2009).

Bayes' Confidence

NEITHER THE PERSPECTIVE "BAYES' THEOREM in the 21st century" (B. Efron, 7 June, p. 1177) nor the responding Letter "A statistically significant future for Bayes' rule" (R. van Hulst, 26 July, p. 343) refer to the mystical flavor often associated with Bayes in their discussions of the theorem's popularity.

Bayes had a propensity to use names that suggest something more than what is directly being described. For example, "Bayes' rule" is just conditional probability applied in a specialized context. The "controversial theorem" is nothing more than a formula for conditional probability.

Perhaps more disconcerting in Bayes is the term "objective prior" for the uninformative priors used by Pierre-Simon Laplace. Such priors, of course, are just imagined; they are not in fact objective themselves, but rather aim to produce objective conclusions. Indeed, many of Laplace's calculations of posterior probability using uninformative priors are numerically equal to frequentist calculations of confidence.

van Hulst mentions that the life sciences need a "synthesis of multiple categories of evidence." Certainly Bayes provides a simple and accessible means of combining different data results: Just multiply the likelihoods together. But this option is also available to the frequentist: Just combine the likelihoods and ignore what's left. The typical frequentist, however, realizes that this method would lose information and is unwilling to make this tradeoff for simplicity. Thus, he would choose an exact confidence interval when available.

Treating Bayes as a route to approximate confidence could go a long way toward resolving the presence of two theories in statistical inference.

D. A. S. FRASER

Department of Statistical Sciences, University of Toronto, Toronto, ON M5S3G3, Canada. E-mail: dfraser@utstat.toronto.edu

Research Funders Should Take the Field

WE SUPPORT EFFORTS TOWARD "LEVELING the playing field" (M. McNutt, Editorial, 26 July, p. 317) in science, technology, engineering, and mathematics (STEM) disciplines through organizations such as the Committee on Women in Science, Engineering, and Medicine (CWSEM). Targeting interventions at early career researchers is vital.

The Wellcome Trust's Basic Scientist Career Tracker (1) demonstrates the disproportionate number of women exiting academia early in their careers. Although an academic research career brings rewards, it remains a risky long-term career choice (2), and as McNutt describes, childbearing years typically coincide with the time when a faculty member needs to build a strong portfolio and gain tenure, thereby securing a less risky future.

Academia needs to attract and retain high-quality, highly trained researchers; research funders such as the Wellcome Trust can play an important role by following these steps: (i) Funders need to ensure that career awareness and mentorship are inte-

gral components of their training provision. (ii) Funders must ensure that their eligibility and/or funding guidelines do not discriminate against certain researchers (for example, a bias in funding decisions toward grant applications that include a move between institutions may inadvertently discriminate against those with established local ties). (iii) Funders need to promote and develop opportunities for researchers to use their funding flexibly, including options for career breaks, reentry fellowships, opportunities to work in posts other than as a principal investigator, and part-time schedules. (iv) We need to expand the opportunities for female role models working across academia to tell their story; this should be a core component of training programs.

ELIZABETH ALLEN,* HALINA SUWALOWSKA,
DAVID LYNN

Strategic Planning and Policy Unit, Wellcome Trust, London, NW1 2BE, UK.

*Corresponding author. E-mail: l.allen@wellcome.ac.uk

Reference

1. Wellcome Trust, "Wellcome Trust Basic Scientist Career Tracker: Results of Wave 4 (2012)" (2013); www.wellcome.ac.uk/Funding/Biomedical-science/Career-tracker/Basic-tracker/index.htm
2. Ipsos MORI, "Risks and rewards: How PhD students choose their careers" (Ipsos MORI, London, 2013).

CORRECTIONS AND CLARIFICATIONS

This Week in Science: "Pushy black hole" (6 September, p. 1041). The last line should be "possibly limiting star formation and galaxy growth" instead of "possibly contributing to star formation and galaxy growth." The HTML and PDF versions online have been corrected.

Reports: "Pandoraviruses: Amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes" by N. Philippe *et al.* (19 July, p. 281). In the first sentence of the legend to Fig. 1, the "(1)" and "(2)" should not have been italicized, as they refer to panels A1/A2 and B1/B2 and not to references 1 and 2. In the legend to Fig. 1E, the "a" and "b" labels should have been transposed. In addition, a reference to panels B1 and B2 is now included. In the acknowledgments, the GenBank accession numbers were incorrectly listed. They should read KC977571 and KC977570 (not KC977471 and KC977470). Also, the financial support of the Provence-Côte-d'Azur Région was missing. The HTML and PDF versions online have been corrected.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

NEUROSCIENCE AND ART

Where Lines Are Drawn

Noah Hutton¹ and Leah Kelly²

This past summer three major U.S. art institutions mounted concurrent retrospectives of James Turrell. For decades, his work has forced us to ask questions about perception. Given recent advances in technology, it would be nice if neuroscience could provide more answers.

Although many recent reports have trumpeted claimed implications of new findings from brain research, an opposing chorus of doubt is surging back [e.g., (1–3)]. The target of such neuroskepticism has often been the frontier outposts of the discipline, where neuroscience pollinates other fields and spawns new monikers such as neuromarketing, neuroeconomics, and neuroaesthetics. At a time when the popularization of using neuroscience to explain everything from love to economics is stirring a backlash, a book about how the brain experiences art could play a critical role in establishing neuroaesthetics as a subject worth taking seriously.

In his preface to *Experiencing Art: In the Brain of the Beholder*, Arthur Shimamura writes that what follows is a “personal account of the ways we experience art.” One wonders whether we are being set up to hear a personal opinion, the voice of an expert, or something in between. Is Shimamura (a psychologist at the University of California, Berkeley) speaking to artists or to other scientists interested in art? He never establishes a firm footing in either direction, leaving one to wonder.

The book reads like a walk through a museum with an author knowledgeable about neuroscience. Shimamura roughly organizes the tour by perceptual faculties: broad chapters on seeing, knowing, and feeling carry the reader through art history—lite tours of major movements, tapping specific works to aid discussions of basic perceptual science. He effectively and comprehensively summarizes the history of neuroaesthetics, illustrating his points using long-established, introductory-level psychology and cognitive science. It’s a familiar routine: most of the artworks discussed here have been pored over several times before by the heavyweights of neuroaesthetics [e.g., (4–6)]. As

a result, *Experiencing Art* treads in all too familiar territory.

The chapters have seductive titles (e.g., “The Eye as Canvas, the Brain as Beholder”), but Shimamura approaches the art history and scientific discourse within them with the sort of cross-disciplinary surface-skimming that only adds fuel to the fires of contemporary academic turf battles. Tangential oversimplifications abound, which feel more amateur than authoritative: “Warhol’s point was to bring the familiar and mundane up to the echelon of high art. The fact that these artworks are valuable and prominently displayed in art museums shows that Warhol succeeded.”

Shimamura paints a similarly oversimplified picture of the brain using diagrams that, for a book about aesthetics, seem especially oblique and confusing. He describes each region as doing its singular, modular task: “We engage the PPC [posterior parietal cortex] when we use our imagination, such as thinking about the future or reminiscing about a past experience.” The author neglects to point out how much we don’t yet know and how poor the resolution of the imaging technology he leans on for his descrip-

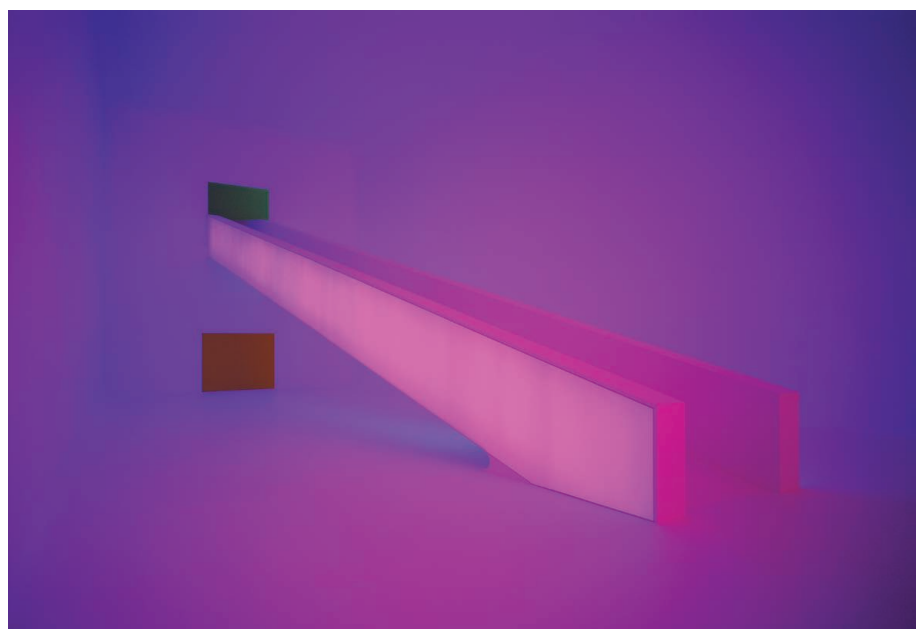
tions is. Nor does he point toward new lines of research ahead. The book’s content and Shimamura’s presentational tone lead us to believe that all the answers are known, neatly available in functional definitions symptomatic of this functional magnetic resonance imaging-heavy era.

Shimamura does propose an integrative model to describe the universal experience of art (I-SKE, for artist’s intention, then the beholder’s sensation, knowledge, and emotion). But it is still too simplistic; he needs to go further. How do knowledge, experience, emotion, and stimuli interact to create a response? For all its limitations, neuroscience is starting to tease these relationships apart. It isn’t clear why, for example, Shimamura omits Jesse Prinz’s recent studies on emotional responses to artworks (7) or Vittorio Gallese’s contributions to the field of embodied cognition (8) from his discussion while recapping classic cases such as H.M. and Phineas Gage.

The current din of territorial squabbles among philosophers, scientists, artists, and art historians makes positive collaborations in neuroaesthetics and the responsible, useful crossing of disciplines all the harder to hear. For the critics, these frontier outposts of a doomed discipline signal the most irresponsible applications of science around today, with excessive reduction and intractable explanatory gaps as the chief concerns. As Noë wrote, “What is striking about neuroaesthetics is ... that it has failed

Experiencing Art
In the Brain of the
Beholder

by Arthur P. Shimamura
Oxford University Press,
Oxford, 2013. 312 pp.
\$49.95, £35.99. ISBN
9780199936939.



James Turrell's *Bridget's Bardo* (2009).

¹<http://thebeautifulbrain.com>. E-mail: noahhutton@gmail.com; ²Laboratory of Molecular Genetics, Rockefeller University, 1230 York Avenue, New York, NY 10065, USA. E-mail: lkelly@rockefeller.edu

to produce interesting or surprising results about art" (2).

One would have hoped, then, that *Experiencing Art* had presented its arguments in a sensitive manner, acknowledging the sharply critical climate it faces. Neuroaesthetics needs responsible advocates who can bring to light the contributions that scientific research undoubtedly has and will continue to bear for art theory and art history, and perhaps vice versa. In the meantime, let us be wary of the expense of bridges built where there is no new ground to be covered.

References

1. P. Legrenzi, C. Umiltà, *Neuromania: On the Limits of Brain Science*, F. Anderson, Transl. (Oxford Univ. Press, Oxford, 2011).
2. A. Noë, <http://opinionator.blogs.nytimes.com/2011/12/04/art-and-the-limits-of-neuroscience/>.
3. S. Satel, S. O. Lilienfeld, *Brainwashed: The Seductive Appeal of Mindless Neuroscience* (Basic, New York, 2013).
4. S. Zeki, *Inner Vision: An Exploration of Art and the Brain* (Oxford Univ. Press, Oxford, 1999).
5. M. Livingstone, *Vision and Art: The Biology of Seeing* (Abrams, New York, 2002).
6. V. S. Ramachandran, *The Tell-Tale Brain: A Neuroscientist's Quest for What Makes Us Human* (Norton, New York, 2012).
7. K. J. Eskine, N. A. Kaciniuk, J. J. Prinz, *Emotion* **12**, 1071 (2012).
8. V. Gallese, C. Sinigaglia, *Trends Cogn. Sci.* **15**, 512 (2011).

10.1126/science.1243937

PSYCHOLOGY

Rewriting Milgram

Ruud Abma

“**T**he experiment requires that you continue, teacher.” It is August 1961. While the “learner” in the next room is begging him to stop, the person filling the role of “teacher” is urged by a stern-looking scientist to keep testing and, if the answer is wrong, administering electric shocks. The teacher had been told that the experiment addressed the effect of punishment on learning, but in reality it was about obedience: with each wrong answer on the memory test, teachers were to increase the voltage. How far would people go? Far, it appeared: 65% of the teachers continued to the possibly deadly level of 450 volts. (In reality, of course, no shocks were given.)

The experiment was one of a series carried out by Stanley Milgram, an ambitious young psychologist at Yale University, who presented the results as evidence that even



Misleading machine. Milgram and the simulated shock machine that he used in his experiments.

ordinary American citizens could be pressed to torture fellow humans. The experiments brought Milgram to center stage in experimental psychology as well as the mass media: He seemed to offer an explanation for the obedience of Nazi officials such as Adolf Eichmann. Moreover, he claimed to have captured a universal truth about human nature: in the face of authority, human conscience is frail.

In *Behind the Shock Machine*, Gina Perry challenges the received view of these experiments. After setting out to produce a story on the subjects who participated in Milgram's study, Perry (an Australian psychologist and writer) discovers a disturbing reality behind the standard account of the experiments. Listening to the audio tapes and studying the Milgram documents in Yale's archives, she found that both the resistance and the distress of the subjects were much greater than suggested by the cold figures Milgram presented. Moreover, a substantial number of the 780 participants did not receive an adequate debriefing, which means they went home not knowing what had really happened.

The experiments, which were run between August 1961 and May 1962, comprised a variety of conditions, many of which did not produce the high levels of obedience that made it into the standard account. Their results depended crucially on the degree of pressure exerted on the subjects. Furthermore, there were subjects who saw through the disguise of the experiments: They could simply not believe that a prestigious university such as Yale would allow its researchers to risk the lives of citizens, whereupon they concluded that the “teachers” were the real subjects—not the “learners.” This shift of

perspective obviously lowered the threshold for administering shocks.

Perry's precise reconstruction of the 24 conditions also cast doubts on the methodological rigor of Milgram's experiments. Rather than testing hypotheses, they were aimed at demonstrating that anyone could be talked into torturing a fellow citizen—a pedagogical lesson for all of us. Making them work this way took a lot of preparation, training, and trial and error. Mil-

gram's students, Perry's interviews revealed, saw him as a “genius” in the designing of the experiments, bringing “art to science.”

Perry also considers what happened to the participants who took part in Milgram's study: Did the experience change their lives?

Were they traumatized by the experience or instead thankful for an increased self-knowledge? Follow-up interviews in 1962–63 had shown a variety of responses—which belied the idea that this complex emotional situation could be reduced to a single outcome measure (i.e., the maximum voltage administered). Perry managed to track down a few

participants. Her interviews with them further undermine Milgram's carefully crafted renderings. Their varied personal interpretations of what had been going on ranged from disbelief in the setup (“I'm not delivering shocks at all”) to anger and grief about the way they were deceived.

Milgram usually advertised his results as “profound and disturbing truths of human nature.” Privately, however, he would acknowledge that his experiments were more successful as drama than as science: “Whether all of this ballyhoo points to significant science or merely effective theater is an open question. I am inclined to accept the latter interpretation.” The merit of *Behind the Shock Machine* is that Perry gives us a thorough look backstage and helps us understand the interpretations and emotions of the actors. Moreover, her elegant and well-written account teaches us that scientists are both investigators and storytellers—and that in both capacities, they should be critically assessed.

10.1126/science.1244504

Behind the Shock Machine

The Untold Story of the Notorious Milgram Psychology Experiments

by Gina Perry

New Press, New York, 2013.

351 pp. \$26.95.

ISBN 9781595589217.

Scribe, Brunswick, Victoria,

Australia, 2012. 432 pp. AU\$32.95.

ISBN 9781921844553.

The reviewer is at the Department of Interdisciplinary Social Science, Utrecht University, Utrecht, Netherlands. E-mail: r.abma@uu.nl

CREDIT: COURTESY ALEXANDRA MILGRAM

SCIENCE EDUCATION

Increasing Persistence of College Students in STEM

Mark J. Graham,^{1,2} Jennifer Frederick,¹ Angela Byars-Winston,³ Anne-Barrie Hunter,⁴ Jo Handelsman^{1*}

A 2012 report by the President's Council of Advisors on Science and Technology (PCAST) predicts that the U.S. workforce will suffer a deficit of one million college graduates in science, technology, engineering, and mathematics (STEM) over the next decade (1). The report calls for addressing the shortfall by increasing retention of college students in STEM. But many academic leaders have not responded aggressively to workforce needs by implementing measures that increase retention. Some of this nonaction is likely due to lack of knowledge about proven retention strategies.

Here, we introduce a "persistence framework" that integrates evidence from psychology and education research into a guide for launching and evaluating initiatives aimed at increasing persistence of interested college students (2–5). We emphasize "persistence," which focuses on student agency (6), rather than on the institutional perspective of "retention," although the intended outcomes are the same. Most of the research we review was conducted in the United States, but the problem is not unique to one country, and the solutions are probably universal.

Persistence of more top students would address the projected STEM workforce deficit, while building a deeper, broader talent pool (1). Less than half of the three million students who enter U.S. colleges yearly intending to major in a STEM field persist in STEM until graduation (1). The exit rate is especially high for the so-called "underrepresented majority," women, racial, and ethnic minorities who are underrepresented in STEM majors but collectively make up 68% of college students in the United States (7). For example, African-American students who intend to major in STEM switch to a non-STEM field before graduation twice as

often as white students (8). Such stark statistics invite a hard look at research and practice that bear on retention.

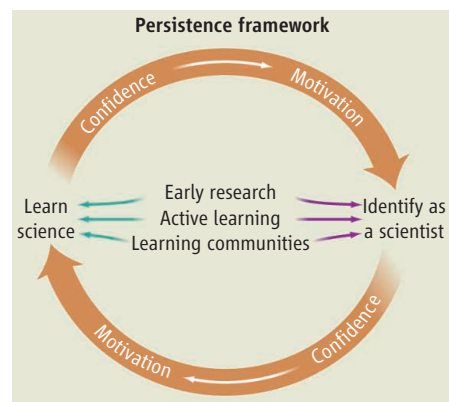
The concept of persistence originates in social and cognitive psychology as one manifestation of motivation (6). In education, motivation is viewed as a driver of student engagement. Among the important constructs underlying motivation is the powerful influence of confidence (i.e., self-efficacy), which is a requirement for persistence (9). Therefore, it is imperative that persistence efforts address motivation and confidence (see the figure).

The framework identifies learning and professional identification as determinants of persistence. Research demonstrates their importance in predicting student behavior (2, 4, 10), and both can be modulated by myriad interventions (7). Some of the most successful STEM retention initiatives pay careful attention to both elements (3). Moreover, both learning and professional identification increase confidence and, consequently, motivation, which in turn spur academic success and feeling like a scientist, thus creating mutually reinforcing experiences (see the figure). This contrasts with student reports of many current introductory STEM courses that obscure the subject, diminish students' confidence, and discourage them from becoming scientists (7). Although the conceptual elements are well established, unifying them into a single persistence framework for guiding STEM education is new.

Answering PCAST's call to increase STEM student retention requires widespread attention. Departments and institutions also need flexibility in the approaches they take and a look at working examples they can model. Because the framework unifies principles that may be implicit in even the most successful programs, we highlight a few intensively studied ones with quantifiable success (although many other successful models exist).

For African-American students and other underrepresented groups, the University of Maryland–Baltimore County Meyerhoff Scholars Program has dramatically increased student achievement, retention, and graduate

An evidence-based framework offers a guide for efforts to increase student persistence in STEM majors.



The Persistence Framework. Confidence is belief in one's own ability; motivation is intention to take action in pursuit of goals; learning is acquiring knowledge and skills; and professional identification is feeling like a scientist.

study in STEM fields. Of their 508 STEM majors between 1993 and 2006, Meyerhoff boasts 86% retention in STEM (3), twice the nationwide average for all students and more than four times the average retention for African-American students. Other programs such as the Biology Scholars Program at University of California, Berkeley (11), more broadly target gender, racial, and ethnic groups. Another approach is the peer-led Gateway Science Workshops at Northwestern University (12), which are open to all beginning STEM students. The Posse programs that focus on urban-schooled science students (1), and the LA-STEM and Howard Hughes Medical Institute (HHMI) Research Scholars Programs at Louisiana State University that focuses on promoting student diversity in STEM are other outstanding examples.

Such successful programs commonly use three interventions widely recognized for inspiring STEM students: (i) early research experiences, (ii) active learning in introductory courses, and (iii) membership in STEM learning communities (see the figure).

Early research experiences. Despite well-known benefits of research experience, most undergraduates are not offered research opportunities until late in college, after the critical period of attrition from STEM

¹Center for Scientific Teaching, Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA. ²Department of Psychiatry, School of Medicine, Yale University, New Haven, CT 06511, USA. ³Department of Medicine, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53705, USA. ⁴Ethnography and Evaluation Research, University of Colorado, Boulder, CO 80309, USA.

*Corresponding author. E-mail: jo.handelsman@yale.edu

majors (13). Students who engage in research in the first 2 years of college are more likely to persist in STEM majors (14). Research experience is a powerful learning tool, engaging students and stimulating curiosity; and it naturally encourages professional identification because students are being scientists, not just studying products of other people's science.

The PCAST report recommends implementing research courses for all beginning undergraduates (1). Research courses provide students with the project ownership and intellectual challenges of empirical pursuit. At the same time, these courses use teaching time and materials efficiently by having student teams work on parallel problems that require similar techniques.

In research courses, students engage in authentic research—they design experiments, collect and analyze data, and sometimes make significant discoveries (15); thus, undergraduates in research courses experience the same dramatic gains in learning and positive attitudes toward science as those who conduct research in faculty laboratories (10, 16).

A variety of research courses have been implemented successfully at large and small institutions. Faculty who are understandably hesitant to accept inexperienced undergraduates into their research laboratories find research courses feasible and rewarding to teach. One is the multi-institutional HHMI–Science Education Alliance (SEA) PHAGES in which freshman discover new bacteriophages from soil (15). The University of Texas at Austin's Freshman Research Initiative demonstrates that research courses can also be cost-effective on a large scale when they replace traditional introductory lab courses. In the Austin model, faculty provide projects, derived from their own research, as the basis for student research projects in lab sections of 20 to 30 students.

Active learning in introductory courses. Many talented college students flee STEM majors because they find introductory courses uninspiring (7). This can be corrected by incorporating classroom teaching practices that engage students in the learning process, known as “active learning,” which has been shown to reduce STEM attrition (17). Active learning includes any activity in which every student must think, create, or solve a problem. For example, brief lectures interspersed with opportunities for students to reflect on or apply their own knowledge

PUTTING THE PERSISTENCE FRAMEWORK INTO ACTION

- (i) Faculty and instructional staff should teach undergraduate research courses, use active learning in introductory STEM courses, and encourage students to form learning communities;
- (ii) Students should be educated about the benefits of learning communities and supported to create their own;
- (iii) Departments should examine curricula and reward structures to incentivize effective teaching, and then align them to enable early research and active learning in introductory courses;
- (iv) Provosts, deans, and chairs should advocate for and dedicate resources to changing classroom practice by creating opportunities for instructors to learn new teaching techniques;
- (v) Public and private funding entities should apply the persistence framework to evaluation of new initiatives in STEM undergraduate education; and,
- (vi) Accreditation agencies should incorporate measurements of STEM persistence into their periodic institutional reviews.

induces active engagement in large lecture courses (18). Active learning improves understanding and retention of concepts and information (1), and it helps students identify as scientists because they participate in scientific thinking with peers who create a scientific community.

Faculty are often reluctant to try active learning because of lack of experience, so it is essential to provide training. Opportunities exist at many universities, professional societies, and in the National Academies Summer Institutes on Undergraduate Science Education, a national program that trains instructors in evidence-based instructional methods (19).

Membership in STEM learning communities. Learning communities are typically virtual or physical structures that provide gathering places or events that enable students to work with and learn from each other (12).

Forming learning communities often requires a small financial investment that produces substantial impacts on student achievement and persistence. Establishing learning communities can be as simple as ensuring that all students have access to a study group outside of class or providing an online environment where students can discuss course content. Learning communities can be constructed in tutoring centers where students congregate by course or discipline, science clubs, or science-based residential communities (12). All of these activities stimulate intellectual growth. Involvement with other students who are aspiring scientists also strengthens professional identity.

To ensure inclusion of all students in learning communities, attention must be paid to being impartial. Students from groups typically underrepresented in science are less likely to form study groups, may be unaware

of the academic benefits of group work outside of class, and confront unintentional biases that may make it challenging to break into established cliques (20). Instructors can reduce this tendency by confronting the issue in class and encouraging students to form and join inclusive groups.

Proven interventions exist, so now it is time for all stakeholders to contribute to increasing student persistence in STEM majors (see table). The elements of the persistence framework are universal and can be tailored for any classroom. In the United States, a concerted effort to implement evidence-based strategies will pay off by advancing the goal of having sufficient STEM college graduates to meet projected workforce needs.

References and Notes

1. PCAST, *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics* (PCAST, Washington, DC, 2012).
2. M. Estrada, A. Woodcock, P. R. Hernandez, P. W. Schultz, *J. Educ. Psychol.* **103**, 206–222 (2011).
3. M. F. Summers, F. A. Hrabowski III, *Science* **311**, 1870–1871 (2006).
4. H. Thiry, S. L. Laursen, A.-B. Hunter, *J. Higher Educ.* **82**, 357–388 (2011).
5. A. Byars-Winston, B. Gutierrez, S. Topp, M. Carnes, *CBE Life Sci. Educ.* **10**, 357–367 (2011).
6. A. Bandura, *Am. Psychol.* **44**, 1175–1184 (1989).
7. E. Seymour, N. M. Hewitt, *Talking About Leaving* (Westview Press, Boulder, CO, 1997).
8. National Academy of Sciences, National Academy of Engineering, Institute of Medicine, *Expanding Underrepresented Minority Participation: America's Science and Technology Talent at the Crossroads* (National Academies Press, Washington, DC, 2011).
9. C. S. Dweck, *Am. Psychol.* **41**, 1040–1048 (1986).
10. D. Lopatto *et al.*, *Science* **322**, 684–685 (2008).
11. J. Matsui, R. Liu, C. M. Kane, *Cell Biol. Educ.* **2**, 117–121 (2003).
12. G. Light, M. Micari, *Making Scientists* (Harvard Univ. Press, Cambridge, MA, 2013).
13. S. H. Russell, M. P. Hancok, J. McCullough, *Science* **316**, 548–549 (2007).
14. B. A. Nagda, S. R. Gregerman, J. Jonides, W. Von Hippel, J. S. Lerner, *Rev. Higher Educ.* **22**, 55 (1998).
15. G. F. Hatfull *et al.*, *PLoS Genet.* **2**, e92 (2006).
16. A.-B. Hunter, S. L. Laursen, E. Seymour, *Sci. Educ.* **91**, 36–74 (2007).
17. D. C. Haak, J. HilleRisLambers, E. Pitre, S. Freeman, *Science* **332**, 1213–1216 (2011).
18. J. Handelsman, S. Miller, C. Pfund, *Scientific Teaching* (W. H. Freeman, New York, 2007).
19. C. Pfund *et al.*, *Science* **324**, 470–471 (2009).
20. C. A. Moss-Racusin, J. F. Dovidio, V. L. Brescoll, M. J. Graham, J. Handelsman, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 16474–16479 (2012).

Acknowledgments: This work was supported by a grant to J.H. from the HHMI Professors Program and NIH Grant 1R13GM090574-01. We thank E. Baraban and J. Young for comments on an earlier version of this manuscript.

10.1126/science.1240487

ENVIRONMENTAL SCIENCE

Global Change and Mercury

David P. Krabbenhoft¹ and Elsie M. Sunderland²

More than 140 nations recently agreed to a legally binding treaty on reductions in human uses and releases of mercury that will be signed in October of this year. This follows the 2011 rule in the United States that for the first time regulates mercury emissions from electricity-generating utilities. Several decades of scientific research preceded these important regulations. However, the impacts of global change on environmental mercury concentrations and human exposures remain a major uncertainty affecting the potential effectiveness of regulatory activities.

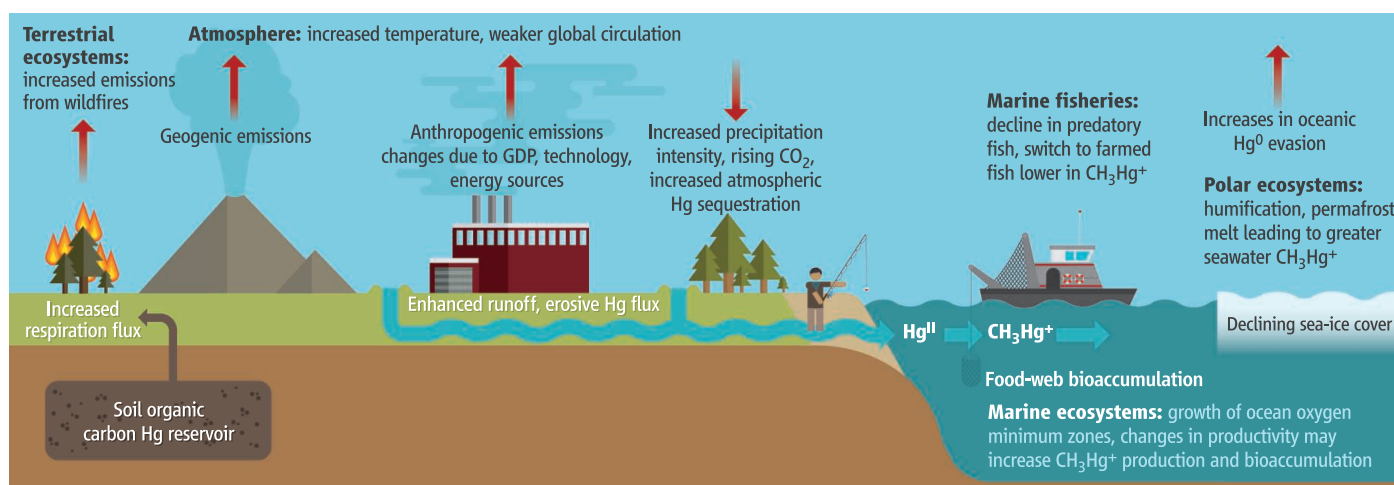
recently replaced coal combustion as the largest anthropogenic mercury emission source globally (3). This often unrecognized industry employs at least 10 to 15 million people globally and poses severe health risks to the miners due to inhalation of concentrated elemental mercury during gold recovery (3).

Most mercury released to the atmosphere is in the gaseous elemental (Hg^0) form, which has a long atmospheric lifetime (6 to 12 months), allowing hemispheric-to-global mixing and transport before deposition. Elemental mercury reacts with atmospheric oxidants such as bromine to form the

Mercury concentrations in the atmosphere and oceans are affected not only by anthropogenic emissions but also by climate and ecosystem change.

Mercury concentrations in the atmosphere and oceans are affected not only by anthropogenic emissions but also by climate and ecosystem change.

Methylation of inorganic mercury in wetlands, lakes, rivers, and seawater leads to the formation of methylmercury, a potent neurotoxin. Methylmercury is the only species of mercury to become concentrated with each successive level in the food chain, reaching levels in predatory fish that are about a million times higher than in seawater (6, 7). Fish are the main methylmercury exposure source for most wildlife and humans. Experimental data



How mercury cycles through the environment. Global change interacts directly and indirectly with mercury cycling. Numerous physical, chemical, and anthropogenic drivers will affect future mercury conditions in the atmosphere, terrestrial systems, and oceans.

Mercury is naturally abundant in heavy-metal-rich geologic deposits and coal. Since antiquity, humans have intentionally (mining) and unintentionally (fossil-fuel combustion) liberated mercury from these stable long-lived reservoirs (1). Global mercury releases increased steeply during the 16th-century silver production rush in Spanish America, and again during the late-19th-century gold rush in North America (2). Over the past century, anthropogenic mercury releases have been dominated by atmospheric emissions from fossil-fuel combustion, particularly coal-fired power plants (2). Artisanal and small-scale gold mining in developing countries has

highly water-soluble divalent mercury species (Hg^{II}) that is rapidly deposited to terrestrial and aquatic ecosystems. Some of this mercury is reduced back to Hg^0 and reemitted to the atmosphere; the remainder cycles through soils and the oceans over time scales ranging from decades to many centuries until it is sequestered in the lithosphere (4).

Sedimentary records of historical pollution provide evidence of a three- to fivefold increase in global atmospheric concentrations since the mid-1800s (5). Assessments that account for releases since antiquity suggest that humans have increased background atmospheric concentrations and deposition by a factor of 7 to 10 worldwide (1, 4). Modeling and observations also provide evidence of human-driven changes in seawater mercury concentrations (4, 6). A shift in global anthropogenic mercury releases from North Amer-

ica and Europe to Asia in recent years can be linked to decadal-scale decreases in mercury concentrations in the Atlantic Ocean and contrasting increases in the Pacific Ocean (6). Epidemiological studies show long-term neurocognitive deficits in children exposed to methylmercury and some evidence for impaired cardiovascular health in adults (9).

Currently, human activities result in mercury emissions of ~2000 metric tons per year. Global anthropogenic emissions scenarios for 2050 range from a best-case decrease from present levels to 800 metric tons per year (driven by mercury-specific control technology and co-benefits from climate mitigation strategies) to an increase to 3400 metric tons per year under a business-as-usual scenario (10, 11). Present-day human sources consti-

¹U.S. Geological Survey, 8505 Research Way, Middleton, WI 53562, USA. ²Department of Environmental Health, Harvard School of Public Health and School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA. E-mail: dpkrabbe@usgs.gov

tute ~30% of atmospheric emissions each year, with the remainder attributed to terrestrial and oceanic emissions that originate from legacy anthropogenic and natural mercury sources (3). Terrestrial and oceanic mercury reservoirs will continue to grow and release more mercury under all but the most stringent emissions controls scenarios, meaning that aggressive emissions reductions are required to stabilize background concentrations in the atmosphere and global oceans (4). However, future emissions trajectories are highly uncertain and depend on changes both in the global economy and in technology penetration.

Global biogeochemical cycling of mercury will also be affected by rapid climate change (see the figure). Weaker global circulation and elevated temperatures will affect atmospheric oxidation rates and patterns of deposition globally. Increased precipitation intensity and incidence of extreme storm events are likely to lead to increased mercury inputs to aquatic systems through direct deposition, runoff, and erosion. Terrestrial soils are the largest global mercury reservoirs (4) and atmospheric mercury sequestration will likely increase in areas where ecosystem primary productivity is stimulated by increases in precipitation and elevated atmospheric CO₂ (12).

Furthermore, increases in the frequency, scale, and intensity of wildfires are likely to mobilize vast stores of mercury in terrestrial soils (4). Changes in ocean circulation, productivity, and growth of oxygen minimum zones will likely alter rates and patterns of methylmercury formation in seawater (6). In polar regions, higher temperatures and declining sea ice could enhance oceanic mercury losses through elevated evasion of Hg⁰. However, changes in primary productivity and species composition in Arctic ice-free waters have also been associated with an increase in biological mercury concentrations due to differences in feeding preferences (13). Many studies have suggested that climate change will exacerbate methylmercury production and bioaccumulation in aquatic ecosystems, but improved understanding of impacts on global mercury biogeochemistry is necessary for anticipating future human and wildlife exposures and risks.

Mercury cycling and bioaccumulation is also affected by other human-driven changes (see the figure). For example, increased ozone concentrations since preindustrial times are thought to have increased the residence time of mercury in the atmosphere by 66% through interactions with bromine (4). This change, coupled with greater emissions, has promoted an overall increase in the worldwide distribution and accumulation of anthropogenic

mercury. Widespread nutrient enrichment of coastal ecosystems is likely to increase methylmercury formation in many ecosystems (6). Increasing consumption of lower-mercury containing fish from aquaculture (particularly in China) and fishing pressure that has decreased the size and trophic level of fish consumed in many regions, have partially offset increases due to rising pollution levels.

The links between environmental mercury cycling and major global change drivers (warming, hydrology, and emission controls) are reasonably understood. However, it remains challenging to forecast a future environment driven by multiple synergistic and antagonistic drivers operating simultaneously. New and rapidly developing scientific tools such as the application of high-resolution mass spectroscopy to fingerprint sources and key processes (14) and genetic markers for the capacity to methylate mercury (15) will help to improve understanding of the cycling and health impacts of environmental mercury.

References

1. O. Serrano, A. Martinez-Cortizas, M. A. Mateo, H. Biester, R. Bindler, *Global Biogeochem. Cycles* **27**, 21 (2013).
2. D. G. Streets et al., *Environ. Sci. Technol.* **45**, 10485 (2011).
3. U.N. Environment Programme, *Global Mercury Assessment 2013: Sources, Emissions, Releases and Environmental Transport* (UNEP Chemicals Branch, Geneva, Switzerland, 2013).
4. H. M. Amos, D. J. Jacob, D. G. Streets, E. M. Sunderland, *Global Biogeochem. Cycles* **27**, 410 (2013).
5. W. F. Fitzgerald, D. R. Engstrom, R. P. Mason, E. A. Nater, *Environ. Sci. Technol.* **32**, 1 (1998).
6. R. P. Mason et al., *Environ. Res.* **119**, 101 (2012).
7. J. M. Benoit, C. C. Gilmour, A. Heyes, R. P. Mason, C. Miller, *ACS Symp. Ser.* **835**, 262 (2003).
8. R. C. Harris et al., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 16586 (2007).
9. M. R. Karagas et al., *Environ. Health Perspect.* **120**, 799 (2012).
10. P. Rafaj, I. Bertok, J. Cofala, W. Schopp, *Atmos. Environ.* **79**, 472 (2013).
11. D. G. Streets, Q. Zhang, Y. Wu, *Environ. Sci. Technol.* **43**, 2983 (2009).
12. O. Hararuk, D. Obrist, Y. Luo, *Biogeosciences* **10**, 2393 (2013).
13. G. A. Stern et al., *Sci. Total Environ.* **414**, 22 (2012).
14. J. D. Blum et al., *Nat. Geosci.* 10.1038/ngeo1918 (2013).
15. J. M. Parks et al., *Science* **339**, 1332 (2013).

10.1126/science.1242838

MATERIALS SCIENCE

A Coat of Many Functions

Dmitry Shchukin¹ and Helmuth Möhwald²

Smart coatings are designed to be sensitive to various external and internal stimuli, thereby enhancing the surface functionality of materials.

The past decade has seen great interest in the development of smart materials with autonomic functionalities. Among them smart coatings have a special niche, filling the position at the interface between bulk solid substrate and liquid or gaseous external environment. This makes them uniquely well suited for such applications as corrosion protection, detection and delivery of bioactive species, and antifouling. They can provide either autonomic response to fluctuations and variations of the coating integrity (disruption, melting) or stimulated response to changes in the external environment (magnetic or electromagnetic fields). The response action depends on the functionalities that the coatings attain during their preparation. The main challenges are to introduce these improvements, maintain them through all manufacturing

steps and material life cycle, and use them efficiently when demanded.

The development of smart coatings possessing rapid or sustained feedback activity in response to external impacts will be an enabling technology for the fabrication of high-tech products with multifunctional surfaces. In general, the coatings combine passive properties inherited from classical coating design (barrier, color, adhesion) and engineered active parts, which are sensitive to instant or gradual impacts occurring either in the coating matrix (pH changes, cracks) or in the environment surrounding the coating (light, temperature, humidity). After exposure to certain impact(s), the active part of the smart coatings responds in order to restore the coating functionality, thus reducing the negative effect of the impact on the coating (self-healing concept) or launching additional properties of the coating interface (bioactivity, detection). The coatings should also have several passive and active functionalities (e.g., antireflection, antifungal, anticorrosion) exhibiting synergistic effects, thus

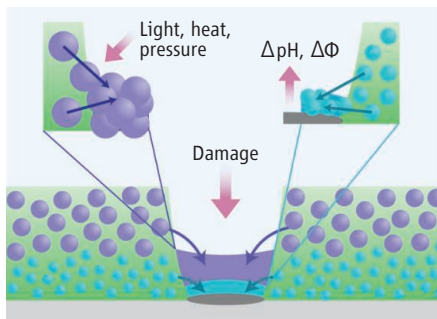
¹Stephenson Institute for Renewable Energy, Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, UK. ²Department of Interfaces, Max Planck Institute of Colloids and Interfaces, D-14476 Golm, Germany. E-mail: d.shchukin@liverpool.ac.uk

rendering the surface of any coated substrate functional and responsive. The key problem is to introduce multiple functions that survive the coating manufacture, do not interact adversely, and preserve their function throughout the coating life cycle.

There are several ways toward a smart and active coating. Besides other methods (vascular, based on tubular networks, or intrinsic, based on mobility of the components of the coating matrix) for design of the active components of smart coatings (1), incorporation of encapsulated active material in the coating matrix is the most promising technology (2, 3). The high versatility of encapsulation technologies, active agents, and shell components allows selection of the appropriate library of nanocontainers with the desired characteristics (size, sensitivity of the shell, compatibility to the matrix), thereby isolating the problems associated with each individual function (see the figure).

The most sophisticated part of smart coatings is the development of nanocontainers with sensitive shells, high loading capacity, and good affinity for the coating matrix. The containers have to be robust, sensitive to external stimuli, and well dispersed in the coating. Being uniformly distributed in the coating, the nanocontainers keep the active material in a trapped state, avoiding undesirable interaction between the active component and the matrix and spontaneous leakage. Each of these parameters is essential for the active property of the smart coatings. The nanocontainer shell becomes sensitive as a result of the weak forces between the shell components during shell formation: electrostatic, pH-bonding, supramolecular, or hydrophobic forces in a layer-by-layer deposition of oppositely interacting species (polyelectrolytes, nanoparticles, biomaterials) on the surface of the capsule scaffold (mesoporous oxide nanoparticle, oil core, polymer shell, etc.) (4). Strong forces such as covalent binding are not sensitive enough to react to weak external stimuli. The following triggers have been shown to be applicable for opening or closing of the container shell: local pH changes, temperature changes, electromagnetic irradiation, mechanical pressure (including ultrasonic treatment), humidity, electric (electrochemical) potential, ionic strength, and dielectric permeability of the solvent.

The simplest trigger for opening or closing of the capsule shell is the pH shift in the local environment. Polyelectrolyte capsules, hydrogels, and emulsions with weak acidic or basic functional groups in the shell can be made sensitive, demonstrating reversible



Nanocontainer-based smart coatings. A fine dispersion of nanocapsules lends active functionality to the coating matrix. Capsules can be loaded with various active materials depending on the functionalities required. The sensitivity of the capsule shell can be adjusted to different stimuli (e.g., pH changes or changes in the electrochemical potential, Φ) for opening and release of the encapsulated active material by nanoengineering of the shell components and structure. [Adapted from (2)]

and/or irreversible changes of the shell permeability in a wide pH range (5). For opening by electromagnetic irradiation, the capsule should have light-sensitive components in the shell: nanoparticles (6) for infrared or ultraviolet light, or dyes for visible light (7). Mechanical impact requires a certain level of rigidity or brittleness of the shell because an elastic shell can undergo deformation under pressure but not rupture (8); containers with diameters of <100 nm can survive reasonable mechanical force because they tend to escape from the force direction.

The smart coating concept has provided effective anticorrosion self-healing coatings and bioactive coatings. Mesoporous nanocontainers (SiO_2 , TiO_2 , CeO_2) and halloysite nanotubes have been used in smart polymer and sol-gel coatings (9, 10). The outer surface of the inorganic containers was coated with pH-sensitive weak polyelectrolytes or other polymers to provide controlled release of the encapsulated inhibitor. The main advantage of the composite inorganic nanocontainers is their small size (typically <200 nm), making them compatible with thin coatings and not affecting mechanical and optical properties of the coatings. The triggering mechanism of inhibitor release is local changes of the pH value caused by the corrosion process, which in turn cause the pH-sensitive polymer or polyelectrolyte shell to open the pores and release encapsulated inhibitor. The main drawback of such containers is the low loading capacity due to the inner mesoporous structure. Clay nanoparticles demonstrated release of encapsulated inhibitors induced by ion-exchange mechanisms (11). Anionic inhibitor was electrostatically bound inside the clay nanoparticles and then exchanged to Cl^- ions in sodium

chloride media. There are also several demonstrations of encapsulation of healing agents or biocides inside nanocontainers with oil interior and polymer shell. Polyurethane microcapsules containing hexamethylene diisocyanate were manufactured via interfacial polymerization of commercial methylene diphenyl diisocyanate prepolymer and 1,4-butanediol in an oil-in-water emulsion (12). Isocyanates are reactive with moisture and can be used as a catalyst-free healing agent. The crack was sealed and healed autonomously to retard the diffusion of salt ions and thus protect the substrate from the corrosion process.

The demonstrated approaches for the fabrication of smart coatings have been great developments for functional organic and composite nanocontainers able to encapsulate active material and release it on demand. However, it remains a challenge to have all functions optimized that would allow the development of a universal approach that combines everything in the one process. Further transfer of these approaches to the technology level will be dictated by the impact of industrial (technical, economic, and ecological) requirements on the coating. Much work still remains in gaining a better understanding of the detailed mechanism of shell permeation and structure of the inner voids. The composition of all elements of the feedback active system has to provide good physicochemical compatibility in terms of such properties as dispersibility and colloidal stability of initial fluid coating formulation, adhesion between containers and host matrix, and adhesion between matrix and substrate in the cured state. Demonstrating the approach with one type of coating with high economic impact, in addition to preserving raw material, will open routes to many other functionalities and applications.

References and Notes

1. B. J. Blaiszik *et al.*, *Annu. Rev. Mater. Res.* **40**, 179 (2010).
2. D. G. Shchukin, H. Möhwald, *Small* **3**, 926 (2007).
3. S. R. White *et al.*, *Science* **409**, 794 (2001).
4. G. Schneider, G. Decher, *Nano Lett.* **4**, 1833 (2004).
5. D. G. Shchukin, G. B. Sukhorukov, *Adv. Mater.* **16**, 671 (2004).
6. A. Muñoz Javier *et al.*, *Langmuir* **24**, 12517 (2008).
7. X. Tao, J. Li, H. Möhwald, *Chemistry* **10**, 3397 (2004).
8. T. A. Kolesnikova *et al.*, *Adv. Funct. Mater.* **20**, 1189 (2010).
9. D. Borisova *et al.*, *ACS Appl. Mater. Interfaces* **5**, 80 (2013).
10. I. A. Kartsonakis, A. C. Balaskas, E. P. Koumoulos, C. A. Charitidis, G. Kordas, *Corros. Sci.* **65**, 481 (2012).
11. J. Tedim *et al.*, *ACS Appl. Mater. Interfaces* **2**, 1528 (2010).
12. M. Huang, J. Yang, *J. Mater. Chem.* **21**, 11123 (2011).

Acknowledgments: Supported by the European Union's 7th Framework Programme (project "MUST"—Multilevel protection of materials for vehicles by smart nanocontainers), and the German Ministry for Education and Research (BMBF) under the ForMat and NanoFutur programmes.

10.1126/science.1242895

Sources of Antimicrobial Resistance

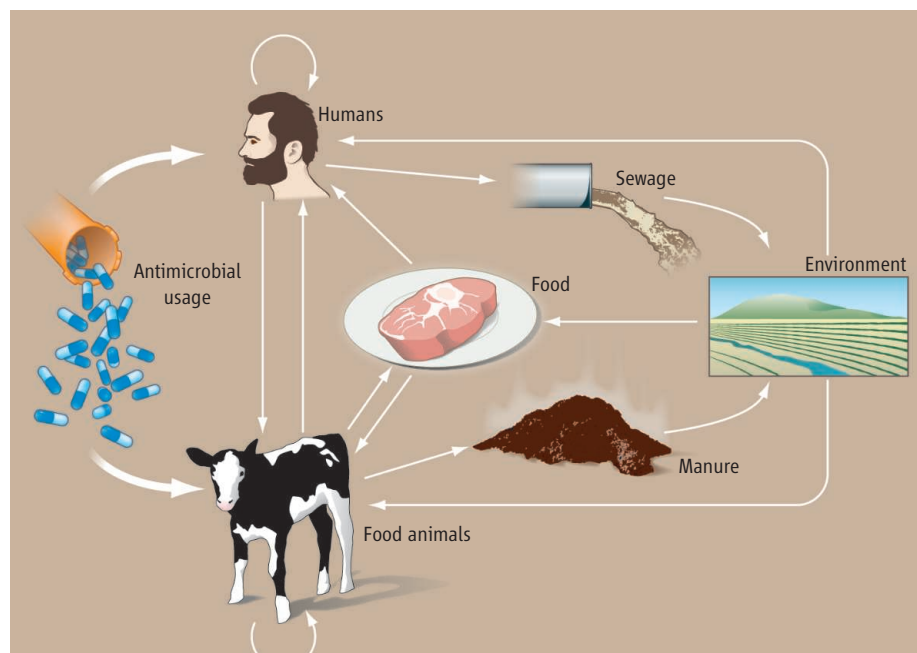
Mark E. J. Woolhouse and Melissa J. Ward

The relentless rise in levels of antimicrobial resistance is an unfolding global public health crisis (1). Resistance to frontline antimicrobials such as fluoroquinolones, third- and fourth-generation cephalosporins, and carbapenems is a particular concern, as is multidrug resistance. The antimicrobial resistance problem is not confined to human medicine: Comparable quantities of antimicrobials are used in livestock production, and resistance is rife in that setting, too, even on organic farms that restrict drug usage (2). Such observations have led to debate about whether antimicrobial resistance in farm animals is an important source of antimicrobial resistance in humans (3, 4). On page 1514 of this issue, Mather *et al.* (5) shed light on this important question in the context of *Salmonella* Typhimurium DT104 in humans and cattle in Scotland.

Antimicrobial resistance can spread from food animals to humans through a number of routes (see the figure). Direct contact with livestock is an important route of exposure for agricultural workers, but the most likely sources of exposure for the general population are thought to be resistant bacteria in livestock-derived food products and animal waste, which is used as a fertilizer of crops and can contaminate water supplies. Yet, antimicrobials are used in very large quantities in the human population, and bacteria carrying resistance genes can spread directly within human populations or indirectly through sewage contamination of food, water, or the wider environment. It is not clear how important the livestock-derived routes are compared with the human-derived routes.

In the 1990s, there was a global epidemic of *S. Typhimurium* DT104. These bacteria are multidrug resistant and are regarded as zoonotic, circulating in the domestic cattle population and acquired by humans mainly by way of food products from cattle—apparently a clear example of the animal-derived route. Mather *et al.* tested this idea for DT104 in Scotland. They conducted whole-genome

Genomic data help to elucidate the role of food animals in the spread of antimicrobial resistance in humans.



Pathways to antimicrobial resistance. Antimicrobial resistance may spread through multiple direct and indirect pathways to humans and food animals (arrows). The relative strength of these pathways will differ greatly not only for different bacteria and different kinds of resistance but also in different locations and environments. Mather *et al.*'s study contributes to the development of a quantitative understanding of this complex process.

sequencing on more than 200 isolates collected by the Scottish Salmonella Reference Laboratory. The isolates were from both humans and livestock, collected over the same 20-year time period and from the same geographical region. Such well-structured samples are rare and valuable resources for epidemiological research.

The genetic sequence data were analyzed using a state-of-the-art approach to elucidate the histories of DT104 in human and animal hosts. The results suggest that the human and livestock epidemics were largely independent, though with some jumps in both directions between the two populations. These findings apply to the DT104 bacterium itself, but antimicrobial resistance can move by horizontal transfer between bacteria. Mather *et al.* therefore investigated the distribution of different multidrug resistance profiles across the DT104 phylogeny. They found more than 30 unique combinations of resistance to individual antimicrobials. The diversity of antimicrobial resistance profiles was at least as high in

the human isolates as in the livestock isolates, and there was little relationship between individual profiles and the phylogeny of the bacteria or the origin of the isolates.

Taken together, the results do not support the hypothesis that the human population of Scotland acquired all their diversity of multidrug-resistant DT104 directly or indirectly from Scottish livestock. So where did it come from? The source is likely to be different DT104s introduced from outside Scotland, particularly in imported food products, with some circulation of these introduced DT104s in the human population.

This expectation can, in principle, be tested in a similar study on a larger geographic scale. However, as Mather *et al.* point out, surveillance and isolate collection outside Scotland have been too patchy to attempt this. This problem is not confined to DT104. The lack of good-quality surveillance data and of access to biological material internationally limits further research on antimicrobial resistance more widely, too.

Centre for Immunology, Infection and Evolution, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JT, UK. E-mail: mark.woolhouse@ed.ac.uk

An interesting corollary of Mather *et al.*'s results is that some of the genetic and antimicrobial resistance diversity of DT104 in Scottish livestock could have been derived from the human population, the opposite of naïve expectation. Yet there is evidence that bacteria do spread from humans to animals. For example, it has been reported that one lineage of the human pathogen *Staphylococcus aureus* (ST5) spread from humans to poultry (6), and another (CC398) to pigs (7), and hundreds of other bacterial species are known to be shared between humans and livestock (8). Given that most antimicrobial resistance is encoded by mobile genetic elements that permit horizontal transfer between different species of bacteria, there is obvious potential for antimicrobial resistance to move quickly and easily in both directions.

The development of antimicrobial resistance cannot be stopped. It is a natural and

ancient phenomenon that originated independently of either modern medicine or industrialized agriculture (9) and is ubiquitous (10). But can the spread of antimicrobial resistance be slowed? Concerted action will be crucial. Antimicrobial resistance is not just a multidrug, multibug problem (3); it is a multihost, multicountry problem as well. The European Union has been pressing its member states to tackle this issue for many years. This year, in response, the UK Department of Health has published a 5-year antimicrobial resistance action plan (4) that will target veterinarians and farmers as well as clinicians and their patients.

This kind of integrated approach is very welcome. However, the epidemiology of antimicrobial resistance is complex (see the figure) and our understanding of it is largely qualitative (11), making it difficult to provide clear advice to policy-makers. We urgently need more comprehensive, rigorous, quanti-

tative studies such as that by Mather *et al.* to know where best to direct our efforts to deal with this pressing problem.

References

1. G. D. Wright, *BMC Biol.* **11**, 51 (2013).
2. D. V. Hoyle *et al.*, *Vet. Microbiol.* **115**, 250 (2006).
3. E. K. Silbergeld, J. Graham, L. B. Price, *Annu. Rev. Public Health* **29**, 151 (2008).
4. Department of Health/Department for Environment, Food and Rural Affairs, *UK Five Year Antimicrobial Resistance Strategy 2013 to 2018* (2013); <https://www.gov.uk/government/publications/uk-5-year-antimicrobial-resistance-strategy-2013-to-2018>.
5. A. E. Mather *et al.*, *Science* **341**, 1514 (2013); 10.1126/science.1240578.
6. B. V. Lowder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 19545 (2009).
7. L. B. Price *et al.*, *mBiol.* **3**, e00305-11 (2012).
8. S. Cleaveland, M. K. Laurenson, L. H. Taylor, *Philos. Trans. R. Soc. Lond. B* **356**, 991 (2001).
9. V. M. D'Costa *et al.*, *Nature* **477**, 457 (2011).
10. K. J. Forsberg *et al.*, *Science* **337**, 1107 (2012).
11. H. C. Davison *et al.*, *Trends Microbiol.* **8**, 554 (2000).

Published online 12 September 2013;
10.1126/science.1243444

MATERIALS SCIENCE

A New Route for Growing Large Grains in Metals

Eric M. Taleff and Nicholas A. Pedrazas

Most metallic materials consist of a network of small single crystals, or grains, connected by grain boundaries. This microstructure, which spans length scales from a few nanometers to hundreds of micrometers, controls many of the properties of the metal. Mechanical processing and thermal treatments can be used to alter this microstructure, but the evolution of grains during processing of a material is governed by phenomena that are so complex (relative to our present scientific understanding) that the outcome cannot be reliably predicted. On page 1500 of this issue, Omori *et al.* (1) describe a wholly unexpected microstructure that arises from synergies among multiple phenomena. They created very large grains in a copper-based shape-memory alloy—a material that will spontaneously recover large strains upon a temperature change—by thermal cycling across temperatures that produce solid-state phase transformations. The subtle mechanisms that apparently act together at elevated temperature to produce this microstructure include internal-stress plasticity

(2) and abnormal grain growth (3). This discovery has potential for technological applications that depend on long service lives of shape-memory alloys.

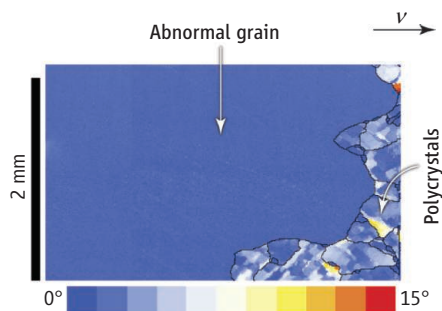
Particular grain structures are desired to achieve specific material properties. Fine grains are generally desired to increase yield strength (which usually will increase the load that the material can bear), but can also be useful for increasing fracture toughness (resistance to cracking) or enabling tremendous ductility at elevated temperatures through superplasticity (4). In shape-memory materials, incompatibility of deformation between grains undergoing the shape-memory transformation can lead to fracturing along grain boundaries and failure after just a few transformation cycles. This is one example of when extremely coarse grains, even single crystals, are desired. Service life may therefore be increased by eliminating the grain boundaries at which deformation incompatibilities might occur. Omori *et al.* created such a microstructure in the copper-aluminum-manganese alloy Cu_{71.6}Al₁₇Mn_{11.4} through abnormal grain growth induced by thermal cycling, a unique discovery. This finding is just one example of how a better understanding of grain growth

Controlled thermal processing can create alloys with large crystalline grains that can lead to improvements in materials performance.

phenomena can enable the engineering of material microstructures for specific applications. Prediction, control, and design of microstructure is a linchpin of current efforts to implement integrated computational materials engineering strategies in manufacturing (5, 6).

Studies of grain growth have shown a natural division into normal and abnormal growth types (3). In normal grain growth, grain sizes increase uniformly throughout a material by the thermally activated migration of grain boundaries. In abnormal grain growth, one or a few grains grow much faster than others. The mechanisms that cause abnormal grain growth are not well understood, and a further complication is that both grain growth types can occur under static and dynamic conditions (7). Most theories of grain growth have been developed for the static case, in which there is not substantial plastic deformation during growth. The phenomena observed by Omori *et al.* suggest a dynamic situation with concurrent plastic deformation generated by internal stresses. The observation of subgrains—partitions within grains produced by the dislocation structures generated during plastic deforma-

Department of Mechanical Engineering, University of Texas, Austin, TX 78712, USA. E-mail: taleff@mail.utexas.edu



Abnormal grain growth. A large abnormal grain grew in a specimen of commercial-purity tantalum (scale bar is 2 mm). Dynamic abnormal grain growth occurred as its grain boundary moved to the right at velocity v when the crystal was heated to 1650°C and pulled along the horizontal direction at a constant true-strain rate of $3 \times 10^{-5} \text{ s}^{-1}$. This large abnormal grain consumed a large region of polycrystalline microstructure during straining and shows little orientation deviation (the image is a map of the relative orientation changes of the crystal axes within each grain). The remaining polycrystalline microstructure shows substantial orientation deviation because of a subgrain structure produced during straining.

tion—verifies this dynamic nature. Abnormal grain growth under such dynamic conditions is perhaps the least studied and understood among grain growth phenomena, yet it produces quite dramatic effects.

The process of abnormal grain growth is illustrated in the figure, which presents an abnormal grain that was grown in commercial-purity tantalum during tensile straining at elevated temperature. The abnormal grain (left) grew into the polycrystalline microstructure (right) by migration of its boundary at some average velocity v . The migration velocity of the boundary is traditionally thought to be a product of the boundary mobility and the thermodynamic driving forces for boundary migration. The figure demonstrates two classical driving forces (8). Boundary curvature drives the boundary of the abnormal grain toward its concave surface into the smaller grains. Reduction of stored strain energy also induces the boundary to migrate into the smaller grains containing subgrains that were produced during straining.

However, questions remain concerning other potential driving forces. For example, there is evidence that applied stress can induce boundary migration (9) in bicrystals (10, 11) and thin films (12), but it is not clear whether stress-driven boundary migration is possible for abnormal grain growth in bulk polycrystalline microstructures where compatibility among many grains is required (13). None of these mechanisms explain why abnormal grain growth proceeds under dynamic conditions when it would have stagnated under static conditions, which is why

dynamic conditions most readily produce the largest abnormal grains (14). It is possible that dynamic conditions alter boundary mobility by affecting local boundary roughening (15), but this avenue of investigation remains largely unexplored.

The combined effect of internal-stress plasticity, subgrain generation, and abnormal grain growth observed by Omori *et al.* adds an exciting new layer to these unsolved mysteries and opens an unexplored path to designing new materials through the control of abnormal grain growth. This discovery also raises new questions, in particular concerning the role of dynamic conditions in boundary mobility and driving forces for grain boundary migration. Past investigations of grain growth phenomena were limited by the absence of techniques for non-destructively characterizing grain structure in bulk specimens. However, new developments such as near-field high-energy x-ray diffraction microscopy (16) may soon enable direct tracking of the evolution of grain structure in bulk specimens.

References and Notes

1. T. Omori *et al.*, *Science* **341**, 1500 (2013).
2. M. Y. Wu, J. Wadsworth, O. D. Sherby, *Metallurg. Trans. A* **18**, 451 (1987).

3. F. J. Humphreys, M. Hatherly, *Recrystallization and Related Annealing Phenomena* (Elsevier Science, New York, ed. 2, 2004), pp. 368–378.
4. T. G. Nieh, J. Wadsworth, O. D. Sherby, *Superplasticity in Metals and Ceramics* (Cambridge Univ. Press, New York, 2007), pp. 40–53.
5. National Research Council, *Integrated Computational Materials Engineering: A Transformational Discipline for Improved Competitiveness and National Security* (National Academies Press, Washington, DC, 2008).
6. Minerals, Metals & Materials Society, *Integrated Computational Materials Engineering (ICME): Implementing ICME in the Aerospace, Automotive and Maritime Industries* (TMS, Warrendale, PA, 2013).
7. J. Ciulik, E. M. Taleff, *Scr. Mater.* **61**, 895 (2009).
8. G. Gottstein, L. S. Shvindlerman, *Grain Boundary Migration in Metals: Thermodynamics, Kinetics, Applications* (CRC Press, Boca Raton, FL, 1999), p. 30.
9. J. W. Cahn, J. E. Taylor, *Acta Mater.* **52**, 4887 (2004).
10. J. Washburn, E. R. Parker, *J. Met.* **4**, 1076 (1952).
11. M. Winning, G. Gottstein, L. S. Shvindlerman, *Acta Mater.* **49**, 211 (2001).
12. T. J. Rupert, D. S. Gianola, U. Gan, K. J. Hemker, *Science* **326**, 1686 (2009).
13. J. W. Cahn, Y. Mishin, A. Suzuki, *Acta Mater.* **54**, 4953 (2006).
14. D. L. Worthington, N. A. Pedrazas, P. J. Noell, E. M. Taleff, *Metallurg. Mater. Trans. A* **10.1007/s11661-013-1865-x** (2013).
15. E. A. Holm, S. M. Foiles, *Science* **328**, 1138 (2010).
16. S. F. Li, R. M. Suter, *J. Appl. Crystallogr.* **46**, 512 (2013).

Acknowledgments: We thank Sandia National Laboratories for the generation of data presented in the figure. Supported by NSF grant DMR-1105468.

10.1126/science.1245056

IMMUNOLOGY

Some Monocytes Got Rhythm

David Druzd and Christoph Scheiermann

Oscillation in the numbers of inflammatory monocytes in different organs depends on a circadian clock gene expressed by myeloid cells.

The molecular mechanisms that control leukocyte migration to sites of inflammation have been a major focus of immunologists over the past 25 years. Recently, an additional dimension has been added to the physiological orchestration of leukocyte trafficking: time. On page 1483 in this issue, Nguyen *et al.* (1) describe a daily oscillation in monocytes present in different mouse tissues, in which there is an increase in number during the animal's time of rest and a decrease during the mouse's active phase. Moreover, this pattern of behavior is governed by a circadian gene in myeloid cells. This suggests that rhythms within this lineage control the

trafficking behavior of monocytes in the organism.

Mouse monocytes are categorized as either “inflammatory” or “resident” on the basis of their migration to various tissues, the potential to differentiate into macrophages and dendritic cells, and the expression of antigens such as Ly6C, chemokine receptor 2 (CCR2), and CX₃C chemokine receptor 1 (CX₃CR1) (2). Macrophages possess an autonomous circadian clock—an intrinsic oscillation in their gene expression pattern over 24 hours (3, 4). In addition, the trafficking behavior of monocytes, neutrophils, and hematopoietic stem and progenitor cells (HSPCs) between blood and tissues oscillates in a rhythmic manner (5). Nguyen *et al.* show that only Ly6C^{hi} “inflammatory” monocytes (those expressing a high amount of Ly6C), but not “resident” monocytes, exhibit a diurnal oscillation that

Walter-Brendel-Center of Experimental Medicine, Ludwig-Maximilians-Universität, 81377 Munich, Germany. E-mail: christoph.scheiermann@med.uni-muenchen.de

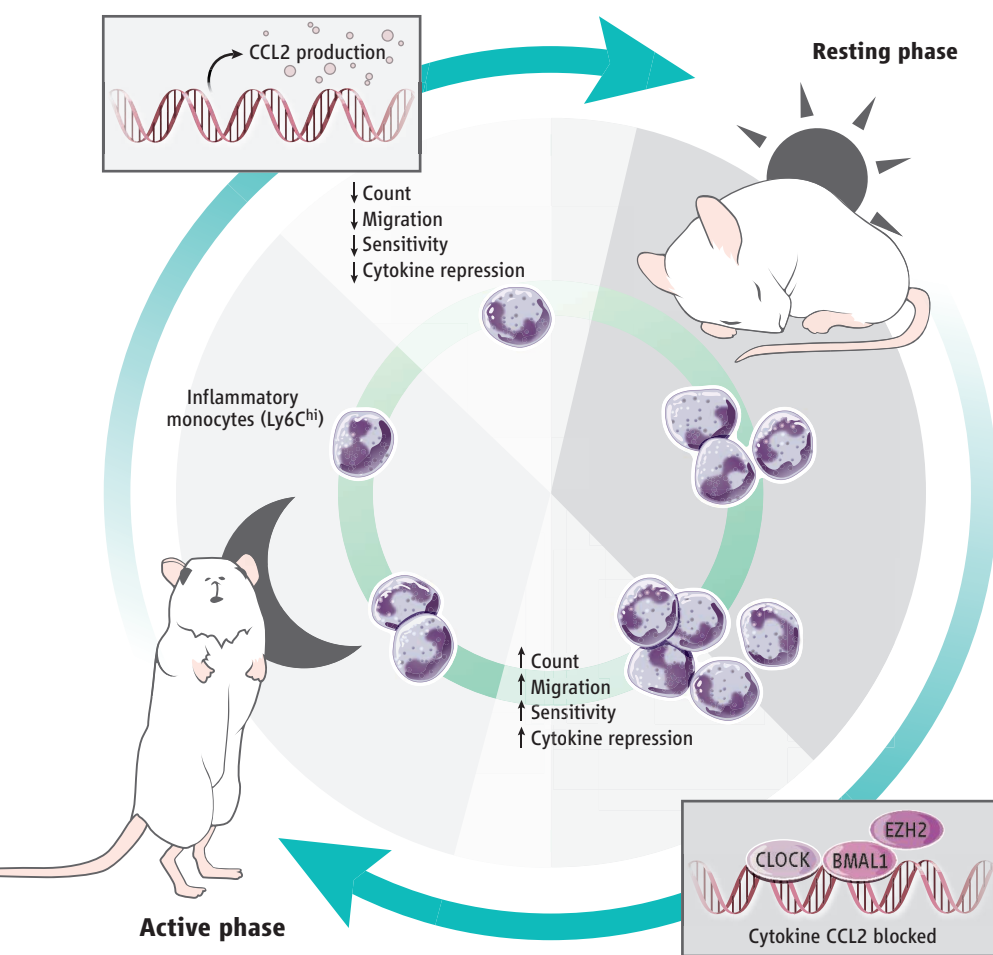
drives rhythmic migratory behavior (see the figure). The authors used genetic methods to deplete the circadian clock gene *Bmal1* in the myeloid lineage and observed that, remarkably, the oscillations ceased. The myeloid population responsible for these effects has yet to be defined. These data are in line with recent findings about rhythms in neutrophil homing to the bone marrow. In the bone marrow, neutrophils are engulfed at the end of their life span by macrophages, stimulating the hematopoietic stem cell niche to mobilize HSPCs into blood (6), a phenomenon that also occurs in a cyclical manner (7). When neutrophil numbers in the circulation were altered by depletion or adoptive transfer, an effect on HSPC mobilization was observed; such modulation of lymphocyte numbers had no effect. Thus, myeloid cells are not mere bystanders

in regulating their numbers, but can modulate the hematopoietic stem cell niche. Circadian variations in immune responses can have dramatic effects, so it is important to understand how different subsets of the myeloid lineage interact rhythmically with each other. The expression of Toll-like receptor (TLR) 9, which plays a fundamental role in pathogen recognition, by splenic macrophages and B cells exhibits a circadian rhythm (8), and vaccination of animals in combination with a synthetic TLR9 adjuvant at the time of peak TLR9 expression enhanced the proliferative adaptive immune response weeks later compared to vaccination at the time of low TLR9 expression. Accordingly, priming the immune response at the right time will likely be a key element in the optimization of vaccination strategies in the future.

A question that arises from recent studies concerns the purpose of oscillatory immune cell trafficking in blood and tissues. Does this happen in anticipation of an expected exposure to pathogen? To address the rhythms in inflammatory monocyte numbers, Nguyen *et al.* used a model of systemic *Listeria monocytogenes* infection. Inoculation of mice at the time when systemic monocyte counts started to decrease—toward the beginning of the mouse's active period—resulted in reduced colony-forming unit content (the number of viable bacteria) in spleen and liver, indicating a correlation between inflammatory monocyte counts and protection against the bacterium. So why do mice fare worse and die earlier when challenged with pathogens at times when the immune response is more sensitive (9)?

The answer probably lies in the amount of pathogen that the organism encounters. Nguyen *et al.* stimulated mice with different amounts of the bacterium toward the beginning of the mouse's active period. Whereas the response to a lower dose resulted in reduced bacterial burden at this time of higher monocyte sensitivity to infection, treatment with a high dose at the same time increased mortality of the infected animals. Because the amount of viable bacteria recovered from organs was the same in both cases, the host's immune response was responsible for the difference in survival. Thus, there appears to be a threshold at which the immune response overreacts, with ensuing life-threatening systemic inflammation or septic shock as a consequence. This suggests that the higher sensitivity of immune cells to pathogens at certain times developed to counter enhanced physiological antigen exposure, which is potentially more likely during the mouse's activity phase. Thus far, this hypothesis lacks experimental proof in mammals. However, for the plant *Arabidopsis thaliana*, naturally occurring cyclical encounter of antigen from the parasite *Hyaloperonospora arabidopsidis* drives rhythmic defense mechanisms (10). This suggests that in host-pathogen interactions, the more mobile organism dictates the timing of the encounter and thereby drives the rhythm in the immune response of the other.

A key observation of Nguyen *et al.* is that BMAL1 in myeloid cells strongly suppresses inflammation. The authors show that under steady-state conditions, BMAL1 binds rhythmically to the promoter regions of *Ccl2* and *Ccl8*, genes that encode proinflammatory cytokines. Interestingly, however, the effect is anti-inflammatory because BMAL1 recruits the histone methyltransferase enhancer of zeste homolog 2 (EZH2)



Timing. “Inflammatory” Ly6C^{hi} monocytes in mice exhibit diurnal oscillations in their trafficking behavior that are controlled by the circadian gene *Bmal1*. At the beginning of the resting phase (daytime for a mouse), the circulating Ly6C^{hi} monocyte count is low as is infiltration into tissues. Expression of the proinflammatory cytokine CCL2 is not suppressed. Toward the animal's active phase (nighttime), the number of circulating Ly6C^{hi} monocytes increases as does their capacity to infiltrate tissues. At the peak of their immune sensitivity to infectious pathogens (toward the beginning of night, when the animal is active), the heterodimeric transcription factors CLOCK and BMAL1 bind to the promoter region of the *Ccl2* gene and rhythmically decrease the production of this cytokine through epigenetic silencing of the locus by the methyltransferase EZH2.

CREDIT: V. ATOUNIAN/SCIENCE

to these genes, which results in epigenetic silencing of the loci. By contrast, in a lethal encounter with pathogen or when BMAL1 is absent in the myeloid lineage, this inhibitory effect is ablated, resulting in increased systemic cytokine expression and dramatically reduced survival in response to infection with *L. monocytogenes*. Thus, rhythmic BMAL1 repression of cytokines at peak sensitivity of the immune response is critical to down-

modulate the inflammatory reaction. Further studies will be necessary to define the precise rhythmic sequence of the complex molecular events that mediate these effects.

References

1. K. D. Nguyen *et al.*, *Science* **341**, 1483 (2013); 10.1126/science.1240636.
2. F. Geissmann *et al.*, *Science* **327**, 656 (2010).
3. J. E. Gibbs *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 582 (2012).

4. M. Keller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21407 (2009).
5. C. Scheiermann, Y. Kunisaki, P. S. Frenette, *Nat. Rev. Immunol.* **13**, 190 (2013).
6. M. Casanova-Acebes *et al.*, *Cell* **153**, 1025 (2013).
7. S. Méndez-Ferrer, D. Lucas, M. Battista, P. S. Frenette, *Nature* **452**, 442 (2008).
8. A. C. Silver *et al.*, *Immunity* **36**, 251 (2012).
9. F. Halberg, E. A. Johnson, B. W. Brown, J. J. Bittner, *Proc. Soc. Exp. Biol. Med.* **103**, 142 (1960).
10. W. Wang *et al.*, *Nature* **470**, 110 (2011).

10.1126/science.1244445

MATERIALS SCIENCE

Small Volumes Create Super(elastic) Effects

Katherine T. Faber

Anyone who has ever broken a favorite coffee cup knows that ceramic materials do not accommodate strain well. Ceramic materials typically fail at strains (extensions) of less than 0.1%. On page 1505 of this issue, Lai *et al.* (1) demonstrate a class of ceramics that can deform reversibly by more than 7%, rendering these solids “superelastic.” To put this in context, at 7% strain, an aluminum can would crumple with no hope of recovery. When Lai *et al.* made small chemical changes to their ceramics, they found a memory effect, whereby the solid can be permanently deformed, but upon heating reverts back to its original size and shape.

Two requirements must be met to produce these effects in ceramics. First, the ceramic compound must undergo a diffusionless (martensitic) crystallographic phase transformation induced by an applied stress or force. Martensitic transformations in ZrO₂-based ceramics—the subject of Lai *et al.*’s study—were recognized nearly 40 years ago (2). The tetragonal-to-monoclinic transformation in these solids is accompanied by changes in volume and shape, a necessary component for superelastic and shape-memory behavior. However, these changes produce volumetric and shear strains that can lead to microcracking and unrecoverable damage (3, 4).

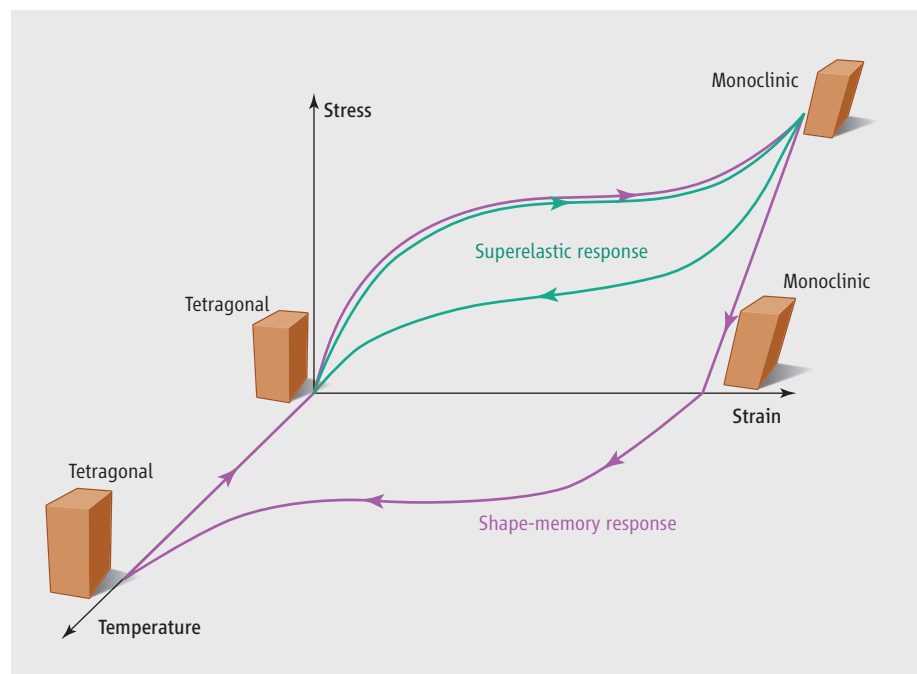
The second requirement, which obviates the microcracking problem, is what highlights the innovation in Lai *et al.*’s work. The sample volume must be reduced to provide a high ratio of surface area to volume, and the

reduced volume must incorporate only a small number of grains. Lai *et al.* were able to meet this requirement with machined pillars of micrometer proportion. They suggest that the high surface area in these small oligocrystalline (few-crystal) volumes provides a source of strain relaxation from the transformation-associated shear strains, preventing damage and allowing for complete reversibility.

Depending on the composition of the ceramic, and thus the temperatures at which crystallographic transformations start and

Superelastic and shape-memory ceramics may find application in high-temperature actuators and energy-harvesting devices.

finish, these phase-transforming solids will function in the superelastic or shape-memory regime (see the figure). For example, if a force is applied to a ZrO₂-based material above its tetragonal transformation temperature, the material can be cycled along the green circuit between tetragonal and monoclinic structures. Energy is dissipated in the form of heat during the loading-unloading cycle; the size of the hysteresis provides a quantitative measure of heat expended in each cycle. The change from a superelastic solid to a shape-memory solid



Making ceramics remember. Schematic stress-strain responses of Lai *et al.*’s superelastic (green) and shape-memory (purple) oligocrystalline ZrO₂ ceramics, which undergo tetragonal-to-monoclinic transformations when stress is applied. The superelastic response is fully reversible, whereas the shape-memory solid requires heat to return to its tetragonal state.

Department of Materials Science and Engineering, Robert R. McCormick School of Engineering and Applied Science, Northwestern University, Evanston, IL 60208, USA. E-mail: k-faber@northwestern.edu

CREDIT: P. HUEY/SCIENCE

can be achieved with relatively small changes in chemistry to permit operation between tetragonal and monoclinic transition temperatures. The shape-memory tetragonal solid follows the purple circuit, transforming to the monoclinic structure under stress. The monoclinic oligocrystal is retained upon removal of the load; the reverse transformation “memory effect” to the tetragonal crystal is only triggered upon heating.

In the late 1980s, Reyes-Morel *et al.* (5) noted shape memory and superelasticity in CeO₂-stabilized tetragonal ZrO₂ polycrystals. However, during their shape-memory experiments, microcracks developed at strains above 1%. Likewise, in superelastic cycling, the strain could not be fully recovered because of damage, and failure occurred after a half-dozen cycles. Wei *et al.* (6) made similar observations. The timing of Lai *et al.*'s work is fortuitous in that micropillars can now routinely be produced and tested (7, 8); furthermore, micro- and nanoelectromechanical devices have become commonplace and provide an obvious platform for applications (9).

Shape-memory properties and superelasticity in metal alloys, such as Ni-Ti, are well developed and are used in stents, orthodontics, and actuators. Shape-memory and superelastic ceramics now allow shape-memory applications to be extended to high temperatures

and corrosive environments. For ZrO₂-based systems, transition temperatures approach 1200°C, where high-temperature actuators such as gate valves and switches may be possible.

In the superelastic regime, these materials could be used in energy-harvesting devices. Lai *et al.* report average energy expended on cycling as great as 35 MJ m⁻³. In contrast, ceramic piezoelectric BaTiO₃ with domain switching-derived superelasticity dissipates only 60 kJ m⁻³—three orders of magnitude lower (10). Superelastic metals such as NiTi-HfPd expend energies during cycling comparable to those of ZrO₂-based materials, but over a more limited range of transformation temperatures (11). The work output of oligocrystalline ceramics surpasses even that of mechanical systems (1).

Lai *et al.*'s study is sure to bring renewed interest in martensitic phase-transforming ceramics for shape-memory and superelastic applications. Possible phase-transforming ceramic candidates for such studies (12) include materials in the lanthanide series, which have substantial volume and shape changes, as well as some silicates. Fundamental issues relating to crystallographic texture and twinning and deformation of small transforming volumes are also likely to be the focus of future research. In composites at

small scales, shape-memory materials may provide self-healing capability (13).

Although little relief is expected for coffee cups, oligocrystalline ceramics that undergo stress-induced martensitic transformations show promise for making high-temperature shape-memory and superelastic applications viable. As such, they open new design space for shape-memory and energy-harvesting devices.

References

1. A. Lai, Z. Du, C. L. Gan, C. A. Schuh, *Science* **341**, 1505 (2013).
2. R. C. Garvie, R. J. Hannink, R. T. Pascoe, *Nature* **258**, 703 (1975).
3. A. G. Evans, R. M. Cannon, *Acta Metall.* **34**, 761 (1986).
4. D. J. Maglev, R. A. Winholtz, K. T. Faber, *J. Am. Ceram. Soc.* **73**, 1641 (1990).
5. P. E. Reyes-Morel, J.-S. Cherng, I.-W. Chen, *J. Am. Ceram. Soc.* **71**, 648 (1988).
6. Z. G. Wei, R. Sandström, S. Miyasaka, *J. Mater. Sci.* **33**, 3743 (1998).
7. B. Daum *et al.*, *Acta Mater.* **61**, 4996 (2013).
8. C. H. Shih, Y. Katoh, K. J. Leonard, H. Bei, E. Lara-Curzio, *J. Mater. Sci.* **48**, 5219 (2013).
9. H. B. Radousky, H. Liang, *Nanotechnology* **23**, 502001 (2012).
10. Y. W. Li, X. B. Ren, F. X. Li, H. S. Luo, D. N. Fang, *Appl. Phys. Lett.* **102**, 092905 (2013).
11. H. Karaca *et al.*, *Acta Mater.* **61**, 5036 (2013).
12. W. M. Kriven, *J. Am. Ceram. Soc.* **71**, 1021 (1988).
13. M. V. Manuel, in *Self-Healing Materials: Fundamentals, Design Strategies and Applications*, S. Ghosh, Ed. (Wiley, Weinheim, 2009), pp. 251–263.

10.1126/science.1245097

CELL BIOLOGY

Stalemate in the Golgi Battle

Brooke Morriswood and Graham Warren

The Golgi—a series of flattened membrane sacs (cisternae)—lies at the hub of a eukaryotic cell's endomembrane system. The routes taken by transiting cargo and the Golgi's resident enzymes have been a matter of long-standing controversy. The two most popular models—stable cisternae versus cisternal maturation—have attracted support and criticism in almost equal measure (1). Two recent papers, aimed at providing a decisive answer for one of the models, instead offer conflicting interpretations of similar data.

For the stable cisternae model, Golgi cisternae are viewed as static structures containing resident enzymes that modify anterograde-transiting cargo. Transport between cisternae is typically carried out by COPI vesi-

cles (COPI is the specific protein complex that coats these vesicles). Hence, cargo moves but the enzymes stay, though there will be some leakage that requires a salvage process should an enzyme stray beyond the cisterna(e) in which it works. This model does not, however, explain how algal scales or procollagen—both too large to fit within COPI vesicles—nonetheless efficiently traverse the Golgi.

Conversely, the cisternal maturation model contends that Golgi cisternae are dynamic structures containing resident cargo, and form through the fusion of cargo-carrying COPII vesicles with retrograde COPI vesicles carrying Golgi enzymes. The cisternae mature through loss of enzymes to earlier (cis) cisternae and acquisition of enzymes from later (trans) cisternae. Large cargo can readily be accommodated because the cisternae are remodeled around it. The process of cisternal maturation has been directly observed

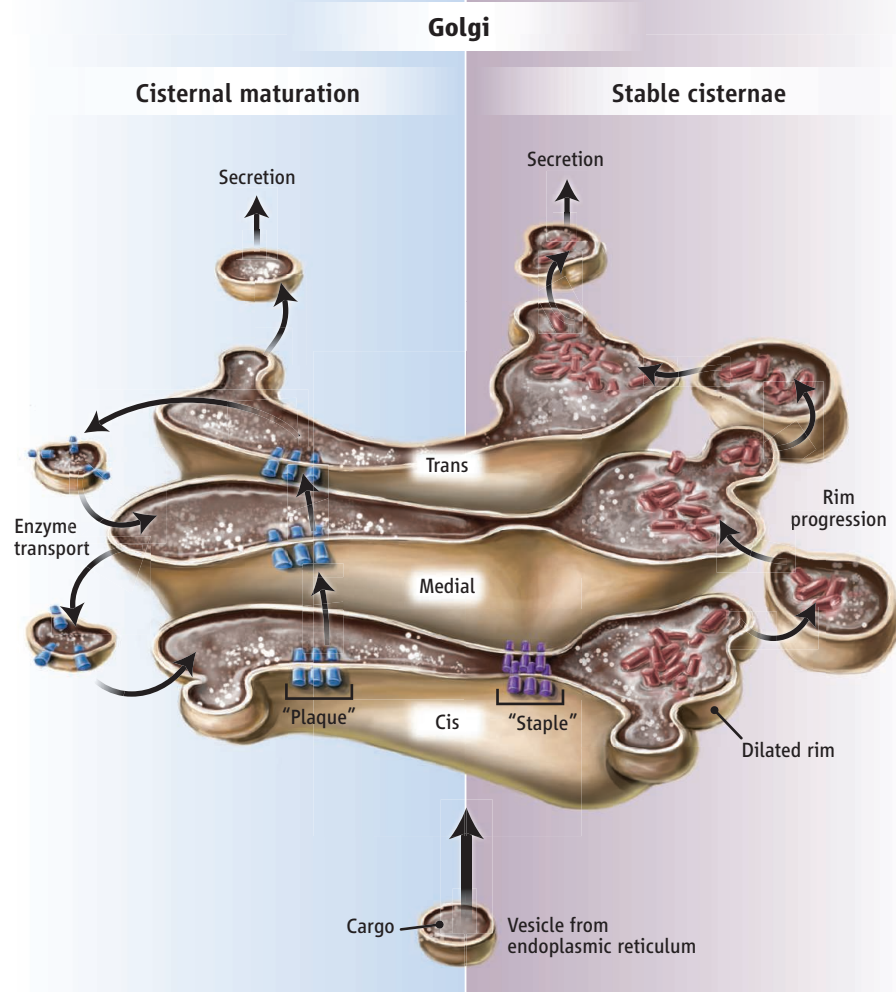
A similar approach to analyze protein trafficking arrives at two opposing models of movement through the cell's secretory pathway.

for Golgi-resident proteins in the unstacked Golgi of budding yeast, although cargo has not yet been simultaneously imaged under the same conditions (2, 3).

The inducible aggregation of reporter proteins offers a novel means of probing these processes, and the two recent studies have exploited this methodology to do so—but with opposing conclusions (4, 5). The method involves fusing a protein of interest to multiple copies of a spontaneously dimerizing module (the F_M domain) (6). Dimerization leads to the formation of aggregates that can be disassembled with a small-molecule ligand, permitting reversible and inducible control of the aggregation process (7).

Lavie *et al.* (4) used this method to study the behavior of membrane-bound secretory cargo. The authors added four copies of the module to the luminal part of an integral membrane protein (CD8) so that it could form

Max F. Perutz Laboratories, Medical University and University of Vienna, Austria. E-mail: graham.warren@mfl.ac.at



Centers and rims. A similar approach for generating protein aggregates provides support for different models of Golgi transport. **(Left)** Membrane-bound Golgi enzyme aggregates (“plaques”) localize to the centers of cisternae and progressively accumulate in the trans-Golgi, consistent with cisternal maturation. **(Right)** Membrane-bound cargo aggregates (“staples”) also localize to the centers of cisternae but do not move, consistent with stable cisternae. Soluble cargo aggregates move by rim progression.

aggregates (called “staples”) that span the cisternal lumen. These aggregates were analyzed with temperature blocks to arrest them at different points along the secretory pathway. When staples were induced in the cis-Golgi, they localized to cisternal centers and could no longer traffic to the trans-Golgi. When staples were induced and trans-Golgi exit blocked, the staples were found throughout the Golgi, indicating that once formed, staples cannot move. In marked contrast, large aggregates of soluble cargo (mimicked by adding the dimerization module to human growth hormone) did move, but localized to the cisternal rims. These results prompted a new model of intra-Golgi transport called “rim progression,” based on an earlier cisternal progenitor model (8).

Rim progression holds that the centers of Golgi cisternae are static and that the rims carry cargo in the anterograde direc-

tion (see the figure). A caveat, however, is that such artificial linking of opposing cisternal membrane creates an unnatural cargo for the Golgi. Therefore, it would be very informative to exploit the observation of Lavie et al. that the membranes of another organelle—the endoplasmic reticulum—can be artificially stacked when the dimerization modules are added to the cytoplasmic tails of reporter proteins (the tails face the cytoplasm and not the lumen of cisternae). If equivalent modules were used to stitch Golgi cisternae to each other, would they too remain in place? Such a result would carry more weight because the stacking of Golgi cisternae is a well-characterized process (9).

Rizzo et al. (5) studied the behavior of Golgi enzymes. Three copies of the dimerization module were added to the membrane-spanning domain (which contains the retention signal) of a cis/medial enzyme

(mannosidase I). In the absence of the small-molecule ligand, the enzyme aggregated to form “plaques” at the centers of cis-Golgi cisternae. Later, the plaques were found in trans-cisternae, implying that they were maturing. Subsequent addition of the small-molecule ligand led to redistribution of the enzyme from the centers to the rims of cisternae and then back to cis/medial cisternae, by way of retrograde vesicles and tubules. These observations are consistent with cisternal maturation. However, retrograde traffic of Golgi enzymes is also a feature of the stable cisternae model, because leaked enzymes must be salvaged.

But why do membrane-bound aggregates of enzyme move through the Golgi, whereas membrane-bound aggregates of cargo do not? One possibility concerns the topology of the aggregates—staples span the cisternal lumen but plaques apparently do not. Why this disparity? It could arise from differences in the number of dimerization modules (four for staples versus three for plaques); the topology of the reporter construct (type 1 versus type 2 orientation in the membrane); the size of the construct (50 versus >100 kD); or the expression level (high versus low). One could readily envision that the staples would be physically excluded from the dilated rims of cisternae, but perhaps the plaques are not and are trafficking by rim progression. This possibility, however, is directly contradicted by Rizzo et al.’s observation that the plaques localize to the centers, and not the rims, of the cisternae.

Hence, we are left with a “central” dilemma: If both types of aggregate are in the same part of the Golgi, how can one move and the other stay behind? More experiments are clearly needed, the key being expression of both reporter proteins in the same cell. So long as they do not form intermingled aggregates, then one can ask whether they move differently. Maybe that answer will finally break the stalemate.

References

1. B. S. Glick, A. Luini, *Cold Spring Harb. Perspect. Biol.* **3**, a005215 (2011).
2. E. Losev et al., *Nature* **441**, 1002 (2006).
3. K. Matsuura-Tokita et al., *Nature* **441**, 1007 (2006).
4. G. Lavie, H. Zheng, J. E. Rothman, *Elife* **2**, e00558 (2013).
5. R. Rizzo et al., *J. Cell Biol.* **201**, 1027 (2013).
6. C. T. Rollins et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7096 (2000).
7. V. M. Rivera et al., *Science* **287**, 826 (2000).
8. S. R. Pfeffer, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19614 (2010).
9. N. Nakamura, J. H. Wei, J. Seemann, *Curr. Opin. Cell Biol.* **24**, 467 (2012).

Published online 19 September 2013;
10.1126/science.1245656

CREDIT: V. AITOUNIAN/SCIENCE

IBI* SERIES WINNER

Students Propose Genetic Solutions to Societal Problems

Sue Wick, Mark Decker, David Matthes, Robin Wright†

In the *Foundations of Biology* sequence for entering biological sciences majors at the University of Minnesota, inquiry-based learning is woven throughout the classroom and laboratory. During the first semester lecture and discussion, students work in teams on a Genetic Engineering Proposal in which they propose a gene-based solution to a societal problem of their own choosing. Instructors coach the teams throughout the semester on experimental design and resources, as well as on data analysis, presentation strategies, team work, and research ethics. On the basis of outcomes from the nearly 3000 students who have taken the course over the past 6 years, the project has succeeded in engaging students in the intellectual work of biologists and the experience of science as creative inquiry.

Our approach emphasizes both scientific teaching (1), an evidence-based approach to course design that applies principles of how people learn (2), and the importance of integrative biology courses (3). We also stress the higher-order skills in Bloom's taxonomy of cognition: synthesis, evaluation, and creation (4). As in the University of Oregon's "Workshop Biology" (5), we encourage student creativity by enabling teams to choose their own project topics. Our course design acknowledges the evidence that team-based learning delivers high learning gains and facilitates development of important life skills (6). This team-based class structure supports the growth of collaborative skills, including giving and receiving

constructive feedback, and necessitates organization, initiative, and communication. As instructors, we are research mentors for teams and coaches for individuals. Moreover, with students in teams of nine working in a SCALE-UP (student-centered active learning environment for undergraduate programs) (7) classroom, we can ask students to accomplish collectively what would be beyond individual capabilities.

The Genetic Engineering Proposal begins with each student in the team identifying a socially important issue for which genetic engineering could provide at least part of the solution, such as bioremediation of contaminated soil by enhanced plants or microbes, improved nutritional value in crops or livestock, or diagnosis and treatment of a disease. After literature searches, team discussions, and feedback from the faculty instructors,

The Genetic Engineering Proposal Project, an IBI prize-winning module, teaches biology students to devise innovative bioproducts or solutions to environmental or health problems.

A team plans their project poster (top); team members at the poster presentations (bottom).

the team settles on a topic that they will jointly pursue for the remainder of the semester (see the top photo). The team must then use primary scientific literature, databases, and, sometimes, interviews with expert researchers to create a compelling argument for the value of the proposed project, to develop an experimental protocol to achieve the end product, and to describe the broader implications of the project, including ethical issues. They must also complete a phylogenetic analysis of their gene of interest or the donor or recipient organisms to address potential methodological problems, environmental impacts, or logical future studies.

Students do not carry out the proposed experiments, which would typically take years to accomplish. However, the proposal enables

students to do the sophisticated intellectual work typical of biologists and explore how science relates to society. They innovate, collaborate, and communicate at a level that transforms their experience of introductory biology and their relationships with one another and with their instructors. They apply what they are learning to solve real-world problems that are important to them and thus experience the relevance of biology concepts. Moreover, the project is inherently integrative, bringing course topics together with information that students locate themselves. Expecting students to begin doing the intellectual work of a biologist inspires them and helps them understand early in their education what a biologist does.

Students have tackled a range of socially important issues [see supplementary materials (SM)]. Even though some project themes



PHOTO CREDITS: (TOP) PAUL BAEPLER; (BOTTOM) PATRICK O'LEARY

University of Minnesota—Twin Cities, St. Paul, MN 55108, USA.

*IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/.

†Corresponding author. E-mail: wrightr@umn.edu

recur, each project is unique and each team's path of inquiry varies. Many projects require students to propose methods not discussed in class or found in their textbook, such as use of retroviruses in gene therapy.

As a result, instructors serve the role of project consultants who also encounter new areas of biology in the process. Thus, the instructors develop collegial relationships with students that demonstrate how biologists work collaboratively in the face of the unknown. Those interactions make this project a highlight for the instructors as well as for the students.

Throughout their work, students maintain intellectual property (IP) notebooks to record the team's conceptual journey and to assure individual accountability. In addition, student evaluations of their own contributions and those of other team members are shared anonymously with the entire team (see SM). The initial formative feedback is used to help team members improve their performance during the semester, and the summative peer evaluation also factors into the individual project grade, as described below, decreasing problems with "free riders."

Consistent with the theme of helping students begin to engage in the professional behaviors of biologists, the culminating poster presentation is similar to those at professional meetings (see the bottom photo). The mood is both intense and celebratory, with students discussing the proposals with rigor and teams enjoying a sense of tremendous accomplishment. Aside from the poster, the completed project includes additional team-created products (a written component, a bibliography, and a critique of another team's written proposal), as well as individual products (a reflection on the project and records of each individual's contributions in the IP notebook; see SM). All components of the project are evaluated by means of detailed evaluation rubrics (see SM) that are provided to students at the beginning of the project. Each individual's grade is subject to decreases based on the evidence provided in the IP notebooks and the final peer evaluation.

Our class sections typically include about 125 students, a number that would create an impossible consultation and/or grading burden for two instructors if students undertook projects individually. The grading workload is reduced to manageable levels because most feedback is given to 14 teams rather than to 125 individuals. In addition, we devote ~40% of class time to project work. Thus, students need to schedule only minimal outside-of-class meetings, and instruc-

About the authors



All are in the College of Biological Sciences at the University of Minnesota. **Sue Wick** (left) is a Morse-Alumni Distinguished Teaching Awardee and professor of Plant Biology and director of undergraduate studies for the Biology Major. Her interests are in fostering inquiry and examining the effectiveness of active learning in kindergarten through grade 16 education. **Mark Decker** (center left) is codirector of and teaching associate professor in the Biology Program. He is interested in the influence that pedagogical approach and use of technology have in developing an understanding of science and critical thinking skills. **David Matthes** (center right) is a teaching associate professor in the Biology Program investigating the function of semaphorin proteins in regulating cell migration in the immune system. **Robin Wright** (right) is a professor of Genetics, Cell Biology, and Development, associate dean for Faculty and Academic Affairs, and a leader within the National Academies Summer Institutes for Undergraduate Biology Education.

tors can provide real-time feedback during class, in addition to evaluating written materials outside of class.

Because the focus is on student collaboration, problem-solving, and active involvement, this type of project is easily adaptable to other courses and institutions. For example, similar projects could be implemented in classes ranging from seminars to large-enrollment courses. Ideally, students would be in active learning classrooms with access to online resources, including databases and primary literature sources, but such access could be via computer labs, personal computers, or classroom-dedicated laptops. The total direct cost of the project is that of printing a poster for each team of nine.

The evidence for project success is apparent in the quality of the products. We evaluate student work with rubrics that include scientific and effective communication criteria. Even with rigorous standards, average project grades are typically between a B+ and A-. Also, the ability of our students to ask significant questions and to propose logical solutions becomes vividly clear when we find industrial, academic, or federal labs pursuing projects similar to those proposed by our students. For example, a team in 2009 planned to genetically modify a patient's cardiac stem cells to treat heart disease; a similar approach was published 2 years later. In 2008, a team proposed to express a squid reflectin gene in microorganisms to incorporate into a camou-

flage suit for military use; in 2012 a publication on recombinant reflectin-based camouflage materials appeared. Most important, in final course evaluations, students consistently rate the project as being of critical importance in achieving learning and development gains in key areas, including scientific reasoning and team work.

References and Notes

1. J. Handelsman, S. Miller, C. Pfund, *Scientific Teaching* (W. H. Freeman, New York, 2007).
2. A. L. Brown, R. R. Cocking, in *How People Learn*, J. D. Bransford, Ed. (National Academy Press, Washington, DC, 2000).
3. National Research Council and NetLibrary, Inc., *BIO 2010: Transforming Undergraduate Education for Future Research Biologists* (National Academy Press, Washington, DC, 2003).
4. L. W. Anderson, D. R. Krathwohl, Eds., *A Taxonomy for Learning, Teaching, and Assessing: A Revision of Bloom's Taxonomy of Educational Objectives* (Longman, New York, 2005).
5. D. Udovic, D. Morris, A. Dickman, J. Postlethwait, P. Wetherwax, *Bioscience* **52**, 272–281 (2002).
6. L. K. Michaelsen, A. B. Knight, L. D. Fink, Eds., *Team-Based Learning: A Transformative Use of Small Groups* (Praeger Publishers, Westport, CT, 2002).
7. R. J. Beichner *et al.*, in *Research-Based Reform of University Physics*, E. F. Redish and P. J. Cooney, Eds. (American Association of Physics Teachers, College Park, MD, 2007), vol. 1, online.

Acknowledgments: We extend our thanks to the thousands of students who have completed Biol 2002 for continuing to demonstrate how much a first-semester biology student can achieve when presented with high expectations.

Supplementary Materials

www.sciencemag.org/content/full/341/6153/1467/suppl/DC1

10.1126/science.1230002

PHOTO CREDITS: S.W.: PATRICK O'LEARY; M.D.: PATRICIA FETTES; D.M. AND R.W.: TIM RUMMELHOFF



EDUCATION

National Effort Spurs Change in Biology Undergraduate Education

“Student-centered teaching” practices such as real-world research experiences and team-based projects clearly help to keep undergraduate students more academically engaged, which may bolster their chances of completing a science-related degree. Yet, research presented at a Washington, D.C., conference co-hosted by AAAS confirmed that faculty in biology and other science-related fields lag behind their peers in pursuing innovative, student-focused teaching methods.

A survey of roughly 23,000 full-time undergraduate teaching faculty at 4-year colleges and universities has shown that faculty in science, technology, engineering, and mathematics (STEM) fields leverage inclusive teaching methods less frequently than their non-STEM counterparts. The survey, directed by Sylvia Hurtado and colleagues with the Higher Education Research Institute (HERI) at the University of California, Los Angeles, was presented at the 28 to 30 August Vision & Change event, spearheaded by the National Science Foundation.

The good news is that biology faculty are “far and away the most likely to involve undergraduates in their research,” said Lorelle L. Espinosa, a senior analyst with Abt Associates who spoke on behalf of Hurtado. But they must do more to see their students through to graduation.

Only about one-third of aspiring biomedical science students complete a bachelor’s degree in the field within 6 years, Espinosa reported. Students—particularly high-achievers—tend to fare better on campuses where faculty have incorporated student-centered teaching practices, said Kevin Eagan, HERI’s assistant director for research. Faculty may be reluctant to reconfigure courses if campus leaders do not seem to value innovative teaching, he said. Others assume that student-centered teaching will not work for introductory courses, although research has debunked this myth.

Improving U.S. biology undergraduate education will be a key to reducing “leakage” in the STEM pipeline, others noted. From a pool of more than 4 million ninth-

Real-world research. The wild marama bean (*Tylosema esculentum*), a legume that could serve as a sustainable crop in subsistence regions, is collected by community workers for a research collaboration between undergraduates at Case Western Reserve University and the University of Namibia. Biology Professor Christopher A. Cullis, who helps the students develop DNA markers for this underutilized crop, said that undergraduate research projects should focus on practical problems so that students must interpret data, rather than merely looking for “correct” answers.

graders in 2001, only 166,530 had earned STEM degrees by 2011, said Muquarrab Qureshi. “That translates into significant gaps for the U.S. Department of Agriculture,” said Qureshi, assistant director of the Institute of Youth, Family, and Community, National Institute of Food and Agriculture (NIFA). “We will have about 54,000 jobs to fill but only 29,000 students who would be able to fill those jobs” between 2010 and 2015.

Continuing national prosperity also will require a more uniformly well-educated population, said Mark Becker, president of Georgia State University. Currently, more than 80% of the most affluent Americans attain a college degree by age 26, compared with only about 8% of those in the bottom quartile of U.S. wealth distribution, Becker said. “It has been reported that in a decade our nation will need approximately 60% of young adults to be college-educated in order for this country to remain competitive with the rest of the world, and that as much as 85% of the new jobs in my metro region will require a bachelor’s degree or greater,” he added. “Right now, only about 30% of all



Mark Becker



Americans have attained a bachelor's degree or greater."

Positive changes are possible. At Georgia State, which has one of the largest populations of low-income students in the nation, Becker reported that 53% of all students now complete a degree within 6 years, up from about 32% in 2003. Moreover, disparities in degree-attainment rates across racial, ethnic, and economic lines have been eliminated. Beginning with a focused, externally reviewed strategic plan, the school collected data, conducted experiments, and scaled up the most effective efforts. For example, peer tutors were deployed to help at-risk students in "high DFW" courses—those in which more than a third of students received a D or an F, or withdrew. Georgia State also expanded freshman learning communities, developed financial interventions, and implemented a centralized, data-driven academic advisement system.

Successful reform efforts require leadership support, and they should address all aspects of the learning environment—from the curriculum and teaching, to how classrooms are configured, said James Collins, who is the Virginia M. Ullman Professor of Natural History and the Environment at Arizona State University: "You can't fix one point on the continuum and expect the continuum to change." If department chairs, deans, provosts, and presidents are not on board, however, Becker said that change can begin when small groups of faculty complete pilot projects: "Find the change leaders. Do the demonstrations. The carping, complaining, and questioning will subside once the data and results speak for themselves."

The Vision & Change conference (www.visionandchange.org) drew 350 attendees from 178 colleges and universities, said Yolanda George, deputy director of AAAS Education and Human Resources. The event was co-hosted by the NSF, NIFA, the Howard Hughes Medical Institute, and the National Institute of General Medical Sciences, which also support a national network of change-focused fellows (www.pulsecommunity.org). Carol Brewer, professor emerita of biology, University of Montana, and AAAS CEO Alan I. Leshner, executive publisher of *Science*, co-chaired the Vision & Change advisory board.

—Ginger Pinholster



INTERNATIONAL

Middle East Seeks to Build Science Capacity

A new agreement between King Abdulaziz City for Science and Technology (KACST) and AAAS's Research Competitiveness Program expands a 5-year-old collaboration that has helped KACST, Saudi Arabia's national science agency, in its efforts "to leapfrog Saudi Arabia toward a knowledge-based society," according to Ahmed M. Alabdulkader, Secretary General for the National Science, Technology and Innovation Plan (NSTIP) at KACST.

The country is one of many across the Middle East and North Africa that aim to develop their scientific output and international collaboration, and are engaging with AAAS to meet some of their strategic goals. While Saudi Arabia and Kuwait have sought hands-on assistance with grant review and administration from the Research Competitiveness Program (RCP), others have participated in more wide-ranging discussions through a meeting series organized by the AAAS Center for Science, Technology and Security Policy (CSTSP).

Since 2008, RCP has managed the peer review of thousands of research proposals submitted to the NSTIP. The analytic reports by AAAS are the next step in achieving "a high level of credibility and reliability of

the national R&D ecosystem," said Alabdulkader. The NSTIP focuses on bolstering national R&D in 15 technology areas, from medicine and health to advanced materials and space, with the goal of making Saudi Arabia a global research power by 2025.

According to the new agreement, signed this year, AAAS will analyze the outcomes of the funded research projects under the NSTIP and the impact of the funds on achieving the country's strategic R&D goals. Now that the submitted proposals have reached a critical mass, the agency wants to "make sure that the projects they are funding are actually proceeding as expected, and that they are able to capture to some extent the great research and educational developments that are bubbling up," said Mark Milutinovich, director of the Research Competitiveness Program.

Building national scientific capacity in this way, through everything from streamlined grant-making to infrastructure and workforce development, is a significant goal for many countries in the Middle East and North Africa, according to an independent report released this month by CSTSP.

The report details the discussions during a unique series of meetings, held



Building a bright future. A field of solar panels at the King Abdulaziz City of Science and Technology, Al-Oyeynah Research Station, demonstrates Saudi Arabia's commitment to strengthening its national science and technology programs to address regional challenges and become a global research power by 2025. Advanced energy technologies are one of 15 focus areas included in the country's National Science, Technology and Innovation Plan.

in Jordan, Kuwait, Tunisia, and the United Arab Emirates between 2010 and 2012, and a cooperative grant program, both facilitated by CSTSP. While the meetings focused on safety and security in practice and in international collaborations in the biological sciences, scientific capacity emerged as a critical and related issue among the broad group of participants. Each meeting drew over 50 scientists, collectively representing 14 countries in the Middle East and North Africa.

Building this capacity and linking it to long-term national plans for science will aid countries in the region as they pursue a broad range of international collaborations as well, the meeting participants concluded. To explore some of these challenges further, the group has launched a BioScience Forum that will meet for the first time in 2014.

As research communities in the Middle East work toward these goals, the Research Competitiveness Program is poised to deliver assistance tailored to a country or university's specific needs. For instance, the program also works on a smaller scale with the Kuwait Foundation for the Advancement of Science, providing advice on grant management and ways to create public-private partnerships for Kuwaiti researchers.

Lessons learned from program clients closer to home have proved useful in international scientific capacity building as well, Milutinovich noted.

"A lot of the work we've done in the past years is tied to U.S. states that have limited resources, and need to think very strategically about how they can leverage their resources and how they can work collaboratively to achieve their aims," Milutinovich said. "So the implementation is going to change, depending on local cultures and the people involved, but the challenges are the same."

—Becky Ham

ELECTIONS

AAAS Annual Election: Preliminary Announcement

The 2013 AAAS election of general and section officers is scheduled to begin in November. All members will receive a ballot for election of the president-elect, members of the Board of Directors, and members of the Committee on Nominations. Members registered in more than one section will receive ballots for elections for each section they are enrolled in.

Candidates are listed below. Additional names may be placed in nomination for any office by petition submitted to the Chief Executive Officer no later than 18 October 2013. Petitions nominating candidates for president-elect, members of the Board, or members of the Committee on Nominations must bear the signatures of at least 100 members of the association. Petitions nominating candidates for any section office must bear the signatures of at least 50 members of the section. A petition to place an additional name in nomination for any office must be accompanied by the nominee's curriculum vitae and statement of acceptance of nomination. Biographical information for the following candidates will be enclosed with the ballots sent to members.

General Election

President-Elect: Lance R. Collins, Cornell Univ.; Geraldine (Geri) Richmond, Univ. of Oregon

Agriculture, Food, and Renewable Resources

Chair Elect: Richard T. Sayre, Los Alamos National Laboratory/New Mexico Consortium; Michael F. Thomashow, Michigan State Univ.

Member-at-Large of the Section Committee: Jerry D. Cohen, Univ. of Minnesota; Elizabeth E. Hood, Arkansas State Univ.

Electorate Nominating Committee: Jim Giovannoni, USDA-ARS; Sheila McCormick, USDA-ARS; Mel Oliver, USDA-ARS; Esther van der Knaap, Ohio State Univ.

Anthropology

Chair Elect: Steven R. Leigh, Univ. of Colorado Boulder; Robert W. Sussman, Washington Univ. in St. Louis

Member-at-Large of the Section Committee: Margaret C. Nelson, Arizona State Univ.; Dawnie Wolfe Steadman, Univ. of Tennessee, Knoxville

Electorate Nominating Committee: Patricia M. Lambert, Utah State Univ.; Lisa J. Lucero, Univ. of Illinois at Urbana-Champaign; Lorena Madrigal, Univ. of South Florida; James J. McKenna, Univ. of Notre Dame

Astronomy

Chair Elect: Stefi Alison Baum, Rochester Institute of Technology; Lee Hartmann, Univ. of Michigan

Member-at-Large of the Section Committee: Jonathan I. Lunine, Cornell Univ.; Jean L. Turner, Univ. of California, Los Angeles

Electorate Nominating Committee: Neil Gehrels, NASA/Univ. of Maryland; Keivan Guadalupe Stassun, Vanderbilt Univ.; Paul A. Vanden Bout, National Radio Astronomy Observatory; Michael Werner, Jet Propulsion Laboratory/Caltech

Atmospheric and Hydrospheric Sciences

Chair Elect: Kelvin K. Droegemeier, Univ. of Oklahoma; Michael J. Prather, Univ. of California, Irvine

Member-at-Large of the Section Committee: Cora E. Randall, Univ. of Colorado Boulder; Soroosh Sorooshian, Univ. of California, Irvine

Electorate Nominating Committee: Lance F. Bosart, Univ. at Albany/SUNY; Anthony J. Broccoli, Rutgers Univ.; Leo J. Donner, NOAA; James A. Yoder, Woods Hole Oceanographic Institution

Council Delegate: David Halpern, Jet Propulsion Laboratory; Richard D. Rosen, NOAA

Biological Sciences

Chair Elect: Steve Henikoff, Fred Hutchinson Cancer Research Center; James T. Kadonaga, Univ. of California, San Diego

Member-at-Large of the Section Committee: Sarah M. (Sally) Assmann, Pennsylvania State Univ.; Susan R. Wente, Vanderbilt Univ. School of Medicine



Electorate Nominating Committee: Vicki L. Chandler, Gordon and Betty Moore Foundation; Claire M. Fraser, Univ. of Maryland School of Medicine; Robb Krumlauf, Stowers Institute for Medical Research; John T. Lis, Cornell Univ.

Chemistry

Chair Elect: Bruce E. Bursten, Univ. of Tennessee, Knoxville; Peter C. Ford, Univ. of California, Santa Barbara

Member-at-Large of the Section Committee: Mahdi Abu-Omar, Purdue Univ.; Mark Thompson, Univ. of Southern California

Electorate Nominating Committee: Philip C. Bevilacqua, Pennsylvania State Univ.; John H. Dawson, Univ. of South Carolina; Katrina M. Miranda, Univ. of Arizona; Galen D. Stucky, Univ. of California, Santa Barbara

Dentistry and Oral Health Sciences

Chair Elect: Adele Ludin Boskey, Cornell Univ./Hospital for Special Surgery; Mina Mina, Univ. of Connecticut Health Center

Member-at-Large of the Section Committee: Rena N. D'Souza, Univ. of Utah School of Dentistry; Van P. Thompson, King's College London Dental Institute (UK)

Electorate Nominating Committee: Pamela K. Den Besten, UC San Francisco School of Dentistry; William Giannobile, Univ. of Michigan; Mark C. Herzberg, Univ. of Minnesota School of Dentistry; Mel L. Kantor, Univ. of Kentucky College of Dentistry and College of Public Health

Council Delegate: Timothy G. Bromage, New York Univ. College of Dentistry; Linda C. Niessen, Texas A&M Univ. Baylor College of Dentistry

Education

Chair Elect: Jay B. Labov, National Academy of Sciences/National Research Council; Martin Storksdieck, National Research Council

Member-at-Large of the Section Committee: David F. Brakke, James Madison Univ.; Tamara Shapiro Ledley, TERC

Electorate Nominating Committee: Margaret R. Caldwell, Center for Ocean Solutions/Stanford Law School; Edna K. DeVore, SETI Institute; Kristin P. Jenkins, Univ. of Wisconsin-Madison/BioQUEST Curriculum Consortium; Alan R. Peterfreund, SageFox Consulting Group

Council Delegate: Katherine Nielsen, Univ. of California, San Francisco; Elizabeth K. Stage, UC Berkeley Lawrence Hall of Science

Engineering

Chair Elect: Richard Alkire, Univ. of Illinois at Urbana-Champaign; W. Kent Fuchs, Cornell Univ.

Member-at-Large of the Section Committee: Nicholas L. Abbott, Univ. of Wisconsin-Madison; Robert M. Kelly, North Carolina State Univ.

Electorate Nominating Committee: Linda Broadbelt, Northwestern Univ.; Surya Mallapragada, Iowa State Univ.; David B. Williams, Ohio State Univ.; Pierre Wiltzius, Univ. of California, Santa Barbara

General Interest in Science and Engineering

Chair Elect: Dominique Brossard, Univ. of Wisconsin-Madison; Dennis Meredith, Independent Consultant

Member-at-Large of the Section Committee: Carol Lynn Alpert, Museum of Science, Boston; Carol L. Rogers, Univ. of Maryland Philip Merrill College of Journalism

Electorate Nominating Committee: Beryl Lief Benderly, Freelance Writer; Jeff Grabmeier, Ohio State Univ.; Robert Lee Hotz, *Wall Street Journal*; Lisa M. Van Pay, George Washington Univ.

Council Delegate: Sharon M. Friedman, Lehigh Univ.; Judith E. Parker, Muhlenberg College/Columbia Univ. Teacher's College

Geology and Geography

Chair Elect: Thorne Lay, Univ. of California, Santa Cruz; Maureen E. Raymo, Lamont-Doherty Earth Observatory

Member-at-Large of the Section Committee: Dennis V. Kent, Rutgers Univ.; Jorge L. Sarmiento, Princeton Univ.

Electorate Nominating Committee: Jeffrey T. Freymueller, Univ. of Alaska Fairbanks; Thomas P. Guilderson, UC Santa Cruz/Lawrence Livermore National Laboratory; Jennifer R. Marlon, Yale Univ.; Konrad (Koni) Steffen, Swiss Federal Research Institute/ETH Zürich (Switzerland)

History and Philosophy of Science

Chair Elect: William Bechtel, Univ. of California, San Diego; Elliott Sober, Univ. of Wisconsin-Madison

Member-at-Large of the Section Committee: Mark E. Borrello, Univ. of Minnesota; Roberta L. Millstein, Univ. of California, Davis

Electorate Nominating Committee: Heather Douglas, Univ. of Waterloo (Canada); Kristine C. Harper, Florida State Univ.; Alan C. Love, Univ. of Minnesota; C. Kenneth Waters, Univ. of Minnesota

Industrial Science and Technology

Chair Elect: Stephen F. Agnew, Columbia Energy and Environmental Services; Sharon C. Glotzer, Univ. of Michigan

Member-at-Large of the Section Committee: Harold H. Kung, Northwestern Univ.; Stephen P. Long, Univ. of Illinois at Urbana-Champaign

Electorate Nominating Committee: Isaac Cann, Univ. of Illinois at Urbana-Champaign; Sang In Lee, Synos Technology, Inc.; Amit Misra, Los Alamos National Laboratory; Michael Tsapatsis, Univ. of Minnesota

Information, Computing, and Communication

Chair Elect: Carla P. Gomes, Cornell Univ.; Eugene H. Spafford, Purdue Univ.

Member-at-Large of the Section Committee: Justine Cassell, Carnegie Mellon Univ.; Boleslaw K. Szymanski, Rensselaer Polytechnic Institute

Electorate Nominating Committee: Bonnie C. Carroll, Information International Associates (IIa)/CENDI; Roscoe C. Giles, Boston Univ.; Tony Hey, Microsoft Research Connections; Susan Landau, Google

Council Delegate: James A. Hendler, Rensselaer Polytechnic Univ.; Karen R. Sollins, Massachusetts Institute of Technology

Linguistics and Language Science

Chair Elect: Brian D. Joseph, Ohio State Univ.; Keren Rice, Univ. of Toronto (Canada)

Member-at-Large of the Section Committee: Wayne O'Neil, Massachusetts Institute of Technology; David Poeppel, New York Univ.



Electorate Nominating Committee: Jonathan David Bobaljik, Univ. of Connecticut; Cecile McKee, Univ. of Arizona; Louise McNally, Universitat Pompeu Fabra (Spain); Shari R. Speer, Ohio State Univ.

Council Delegate: Peter W. Culicover, Ohio State Univ.; Joan Maling, National Science Foundation

Mathematics

Chair Elect: Tony F. Chan, Hong Kong Univ. of Science and Technology; Martin Golubitsky, Ohio State Univ.

Member-at-Large of the Section Committee: L. Pamela (Pam) Cook, Univ. of Delaware; Irene Fonseca, Carnegie Mellon Univ.

Electorate Nominating Committee: Mark Alber, Univ. of Notre Dame; Harvey Thomas Banks, North Carolina State Univ.; Lloyd E. Douglas, Univ. of North Carolina at Greensboro; James (Mac) Hyman, Tulane Univ.

Medical Sciences

Chair Elect: Pamela B. Davis, Case Western Reserve Univ. School of Medicine; W. Ian Lipkin, Columbia Univ./NIAID

Member-at-Large of the Section Committee: Harry (Hal) Dietz, Johns Hopkins Univ. School of Medicine; David M. Sabatini, Massachusetts Institute of Technology

Electorate Nominating Committee: Paul F. Bray, Thomas Jefferson Medical College; Lisa M. Coussens, Oregon Health & Science Univ.; Jules L. Dienstag, Massachusetts General Hospital/Harvard Medical School; Francis W. Luscinskas, Brigham and Women's Hospital/Harvard Medical School; Gregory M. Vercellotti, Univ. of Minnesota

Neuroscience

Chair Elect: Marie-Françoise Chesselet, Univ. of California, Los Angeles; Alison Goate, Washington Univ. School of Medicine in St. Louis

Member-at-Large of the Section Committee: Allan Basbaum, Univ. of California, San Francisco; Robert E. Burke, Columbia Univ.

Electorate Nominating Committee: Suzanne Corkin, MIT/Massachusetts General Hospital; Ruth Anne Eatock, Harvard Univ./Massachusetts Eye & Ear Infirmary; Diane O'Dowd,

Univ. of California, Irvine; Phyllis M. Wise, Univ. of Illinois at Urbana-Champaign

Pharmaceutical Sciences

Chair Elect: David Z. D'Argenio, Univ. of Southern California; Jeanette C. Roberts, Univ. of Wisconsin School of Pharmacy

Member-at-Large of the Section Committee: Donald P. McDonnell, Duke Univ. School of Medicine; Craig K. Svensson, Purdue Univ. College of Pharmacy

Electorate Nominating Committee: Barbara S. Beckman, Tulane Univ. School of Medicine; Carlos Enrique Catalano, Univ. of Washington School of Pharmacy; Melanie S. Joy, Univ. of Colorado School of Medicine and School of Pharmacy; Swati Nagar, Temple Univ.

Council Delegate: David E. Smith, Univ. of Michigan College of Pharmacy; Barbara N. Timmermann, Univ. of Kansas

Physics

Chair Elect: Eva Andrei, Rutgers Univ.; Alexander L. Fetter, Stanford Univ.

Member-at-Large of the Section Committee: Lene Vestergaard Hau, Harvard Univ.; Rolf M. Sinclair, Centro de Estudios Científicos (Chile)

Electorate Nominating Committee: Marcela Carena, Univ. of Chicago/Fermilab; Michael E. Flatté, Univ. of Iowa; Joe D. Thompson, Los Alamos National Laboratory; John M. Tranquada, Brookhaven National Laboratory

Psychology

Chair Elect: Susan Goldin-Meadow, Univ. of Chicago; Nora S. Newcombe, Temple Univ.

Member-at-Large of the Section Committee: Valerie F. Reyna, Cornell Univ./Weill Cornell Medical College; Barbara A. (Bobbie) Spellman, Univ. of Virginia/Univ. of Virginia School of Law

Electorate Nominating Committee: Sian L. Beilock, Univ. of Chicago; Angeline Lillard, Univ. of Virginia; Charles A. Nelson III, Harvard Medical School/Boston Children's Hospital; Elizabeth Phelps, New York Univ.

Social, Economic, and Political Sciences

Chair Elect: Maryann P. Feldman, Univ. of North Carolina at Chapel Hill; Myron P. Gutmann, Univ. of Michigan

Member-at-Large of the Section Committee: Arne L. Kalleberg, Univ. of North Carolina at Chapel Hill; Paula Stephan, Georgia State Univ.

Electorate Nominating Committee: Norman M. Bradburn, National Opinion Research Center at the Univ. of Chicago; Jerald Hage, Univ. of Maryland, College Park; Stephanie Shipp, Virginia Tech; Howard J. Silver, Consortium of Social Science Associations

Societal Impacts of Science and Engineering

Chair Elect: Francesca T. Grifo, Union of Concerned Scientists; Jane Maienschein, Arizona State Univ./Marine Biological Laboratory

Member-at-Large of the Section Committee: Gerald L. Epstein, U.S. Department of Homeland Security; Roger D. Launius, Smithsonian Institution National Air and Space Museum

Electorate Nominating Committee: Jonathan Coopersmith, Texas A&M Univ.; Maryann P. Feldman, Univ. of North Carolina at Chapel Hill; Kevin Finneran, National Academy of Sciences; Tobin L. Smith, Association of American Universities

Council Delegate: Howard Gobstein, Association of Public and Land-grant Universities; Susan Sauer Sloan, National Academies

Statistics

Chair Elect: Sallie Ann Keller, Virginia Tech; Xihong Lin, Harvard School of Public Health

Member-at-Large of the Section Committee: Katherine Bennett Ensor, Rice Univ.; Karen Kafadar, Indiana Univ.

Electorate Nominating Committee: Michael Boehnke, Univ. of Michigan; Dipak K. Dey, Univ. of Connecticut; M. Elizabeth (Betz) Halloran, Univ. of Washington/Fred Hutchinson Cancer Research Center; H.N. Nagaraja, Ohio State Univ.

Council Delegate: Allan R. Sampson, Univ. of Pittsburgh; Linda J. Young, Univ. of Florida/USDA-NASS

Analysis of Surface Materials by the Curiosity Mars Rover

THE 6 AUGUST 2012 ARRIVAL OF THE CURIOSITY ROVER ON THE SURFACE of Mars delivered the most technically advanced geochemistry laboratory ever sent to the surface of another planet. Its 10 instruments (1)* were commissioned for operations and were tested on a diverse set of materials, including rocks, soils, and the atmosphere, during the first 100 martian days (sols) of the mission. The five articles presented in full in the online edition of *Science* (www.sciencemag.org/extra/curiosity), with abstracts in print (pp. 1476–1477), describe the mission's initial results, in which Curiosity's full laboratory capability was used.

Curiosity was sent to explore a site located in Gale crater, where a broad diversity of materials was observed from orbit. Materials representing interactions with aqueous environments were targeted for study because of the emphasis on understanding habitable environments. In addition, the mission's science objectives also include characterizing the geologic diversity of the landing site at all scales, including loose surface materials such as impact ejecta, soils, and windblown accumulations of fine sediments. In certain cases, such characterization may even provide constraints on the evolution of the planet as a whole. Two notable points along Curiosity's initial 500-m traverse included Jake_M, a loose rock sitting on the plains, and Rocknest, an accumulation of windblown sand, silt, and dust that formed in the lee of some rocky outcrops. Sparse outcrops of lithified fluvial conglomerate were also encountered (2).

As described by Stolper *et al.*, Jake_M was encountered ~282 m away from the landing site and is a dark, macroscopically homogeneous igneous rock representing a previously unknown martian magma type. In contrast to the relatively unfractionated Fe-rich and Al-poor tholeiitic basalts typical of martian igneous rocks, it is highly alkaline and fractionated. No other known martian rock is as compositionally similar to terrestrial igneous rocks; Jake_M compares very closely with an uncommon terrestrial rock type known as a mugearite, typically found on ocean islands and in rift zones. It probably originates from magmas generated by low degrees of partial melting at high pressure of possibly water-rich, chemically altered martian mantle that is different from the sources of other known martian basalts.

Over the first 100 sols of the mission, the ChemCam instrument returned >10,000 laser-induced breakdown spectra, helping to characterize surface material diversity. ChemCam's laser acts effectively as a microprobe, distinguishing between fine soil grains and coarser ~1-mm grains. Based on these data, Meslin *et al.* report that the coarse soil fraction contains felsic (Si- and Al-rich) grains, mimicking the composition of larger felsic rock fragments found during the traverse

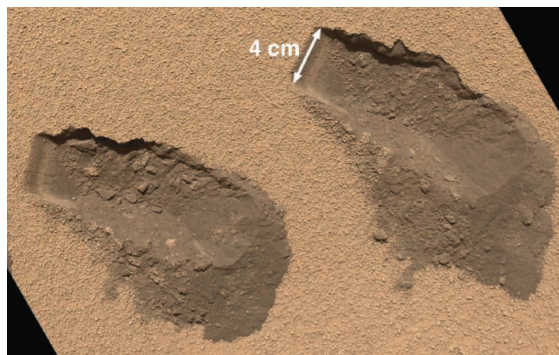
and showing that these larger components probably break apart to form part of the soil. In contrast, the fine-grained soil component is mafic, similar to soils observed by the Pathfinder and Mars Exploration Rover missions.

Curiosity scooped, processed, and analyzed a small deposit of windblown sand/silt/dust at Rocknest that has similar morphology and bulk elemental composition to other aeolian deposits studied at other Mars landing sites. Based solely on analysis of CheMin x-ray diffraction (XRD) data from Mars, calibrated with terrestrial standards, Bish *et al.* estimate the Rocknest deposit to be composed of ~71% crystalline material of basaltic origin, in addition to ~29% x-ray-amorphous materials. In an independent approach, Blake *et al.* used Alpha Particle X-ray Spectrometer data to constrain the bulk composition of the deposit and XRD data and phase stoichiometry to constrain the chemistry of the crystalline component,

with the difference being attributed to the amorphous component, resulting in estimates of ~55% crystalline material of basaltic origin and ~45% x-ray-amorphous materials. The amorphous component may contain nanophase iron oxide similar to what was observed by earlier rovers. The similarity between basaltic soils observed at Rocknest and other Mars sites implies either global-scale mixing of basaltic material or similar regional-scale basaltic source material or some combination of both. No hydrated phases were detected. However, as shown by Leshin *et al.*, pyrolysis of Rocknest fines using the Sample Analysis at Mars (SAM) instrument suite revealed volatile species, probably in the amorphous component, including H₂O, SO₂, CO₂, and

O₂, in order of decreasing abundance. ChemCam measurements of these materials also revealed the presence of H. It is likely that H₂O is contained in the amorphous component and CO₂ was liberated via the decomposition of Fe/Mg carbonates present below the XRD detection limit of 1 to 2%. Isotopic data from SAM indicate that this H₂O, and possibly the CO₂, were derived from the atmosphere. SAM analysis also revealed oxychloride compounds similar to those found by earlier missions, suggesting that their accumulation reflects global planetary processes. The evolution of CO₂ during pyrolysis and the observation of simple chlorohydrocarbons during SAM gas chromatograph mass spectrometer analyses could be consistent with organic carbon derived from a terrestrial instrument background source, or a martian source, either exogenous or indigenous.

—JOHN P. GROTZINGER



Curiosity used its scoop to collect two samples of a small aeolian deposit. The deposit's upper surface is armored by sand grains 0.5 to 1.5 mm in size. These coarse grains are coated with fine dust, giving the deposit an overall light brownish red color. Beneath the coarse sand crust is finer sand, dark brown in color. This Mars Hand Lens Imager image was acquired on sol 84.

Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA.

*References may be found on page 1477 after the abstracts.

10.1126/science.1244258

ABSTRACTS

The Petrochemistry of Jake_M: A Martian Mugearite

E. M. Stolper,* M. B. Baker, M. E. Newcombe, M. E. Schmidt, A. H. Treiman, A. Cousin, M. D. Dyar, M. R. Fisk, R. Gellert, P. L. King, L. Leshin, S. Maurice, S. M. McLennan, M. E. Minitti, G. Perrett, S. Rowland, V. Sautter, R. C. Wiens, MSL Science Team†

*Corresponding author. E-mail: ems@gps.caltech.edu
Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA.

†MSL Science Team authors and affiliations are listed in the supplementary materials. The list of author affiliations is available in the full article online.



"Jake_M," the first rock analyzed by the Alpha Particle X-ray Spectrometer instrument on the Curiosity rover, differs substantially in chemical composition from other known martian igneous rocks: It is alkaline (>15% normative nepheline) and relatively fractionated. Jake_M is compositionally similar to terrestrial mugearites, a rock type typically found at ocean islands and continental rifts. By analogy

with these comparable terrestrial rocks, Jake_M could have been produced by extensive fractional crystallization of a primary alkaline or transitional magma at elevated pressure, with or without elevated water contents. The discovery of Jake_M suggests that alkaline magmas may be more abundant on Mars than on Earth and that Curiosity could encounter even more fractionated alkaline rocks (for example, phonolites and trachytes).

>> Read the full article at <http://dx.doi.org/10.1126/science.1239463>

Soil Diversity and Hydration as Observed by ChemCam at Gale Crater, Mars

P.-Y. Meslin,* O. Gasnault, O. Forni, S. Schröder, A. Cousin, G. Berger, S. M. Clegg, J. Lasue, S. Maurice, V. Sautter, S. Le Mouélic, R. C. Wiens, C. Fabre, W. Goetz, D. Bish, N. Mangold, B. Ehlmann, N. Lanza, A.-M. Harri, R. Anderson, E. Rampe, T. H. McConnochie, P. Pinet, D. Blaney, R. Lévillé, D. Archer, B. Barraclough, S. Bender, D. Blake, J. G. Blank, N. Bridges, B. C. Clark, L. DeFlores, D. Delapp, G. Dromart, M. D. Dyar, M. Fisk, B. Gondet, J. Grotzinger, K. Herkenhoff, J. Johnson, J.-L. Lacour, Y. Langevin, L. Leshin, E. Lewin, M. B. Madsen, N. Melikechi, A. Mezzacappa, M. A. Mischna, J. E. Moores, H. Newsom, A. Ollila, R. Perez, N. Renno, J.-B. Sirven, R. Tokar, M. de la Torre, L. d'Uston, D. Vaniman, A. Yingst, MSL Science Team†

*Corresponding author. E-mail: pmeslin@irap.omp.eu
Université de Toulouse, UPS-OMP, IRAP, 31028 Toulouse, France.
CNRS, IRAP, 9 Av. Colonel Roche, BP 44346, F-31028 Toulouse cedex 4, France.

†MSL Science Team authors and affiliations are listed in the supplementary materials. The list of author affiliations is available in the full article online.



The ChemCam instrument, which provides insight into martian soil chemistry at the submillimeter scale, identified two principal soil types along the Curiosity rover traverse: a fine-grained mafic type and a locally derived, coarse-grained felsic type. The mafic soil component is representative of widespread martian soils and is similar in composition to the martian dust. It possesses a ubiquitous hydrogen signature in ChemCam

spectra, corresponding to the hydration of the amorphous phases found in the soil by the CheMin instrument. This hydration likely accounts for an important fraction of the global hydration of the surface seen by previous orbital measurements. ChemCam analyses did not reveal any significant exchange of water vapor between the regolith and the atmosphere. These observations provide constraints on the nature of the amorphous phases and their hydration.

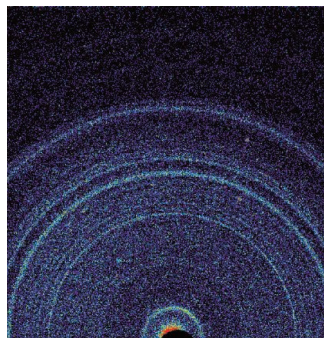
>> Read the full article at <http://dx.doi.org/10.1126/science.1238670>

X-ray Diffraction Results from Mars Science Laboratory: Mineralogy of Rocknest at Gale Crater

D. L. Bish,* D. F. Blake, D. T. Vaniman, S. J. Chipera, R. V. Morris, D. W. Ming, A. H. Treiman, P. Sarrazin, S. M. Morrison, R. T. Downs, C. N. Achilles, A. S. Yen, T. F. Bristow, J. A. Crisp, J. M. Morookian, J. D. Farmer, E. B. Rampe, E. M. Stolper, N. Spanovich, MSL Science Team†

*Corresponding author. E-mail: bish@indiana.edu
Department of Geological Sciences, Indiana University, Bloomington, IN 47405, USA.
†MSL Science Team authors and affiliations are listed in the supplementary materials. The list of author affiliations is available in the full article online.

The Mars Science Laboratory rover Curiosity scooped samples of soil from the Rocknest aeolian bedform in Gale crater. Analysis of the soil with the Chemistry and Mineralogy (CheMin) x-ray diffraction (XRD) instrument revealed plagioclase (~An57), forsteritic olivine (~Fo62), augite, and pigeonite, with minor K-feldspar, magnetite, quartz, anhydrite, hematite, and ilmenite. The minor phases are present at, or near, detection limits. The soil also contains 27 ± 14 weight percent x-ray amorphous material, likely containing multiple Fe³⁺- and volatile-bearing phases, including possibly a substance resembling hisingerite. The crystalline component is similar to the normative mineralogy of certain basaltic rocks from Gusev crater on Mars and of martian basaltic meteorites. The amorphous com-



CREDITS: (LEFT) NASA; (TOP RIGHT) MESLIN ET AL.; (BOTTOM RIGHT) BISH ET AL.

ponent is similar to that found on Earth in places such as soils on the Mauna Kea volcano, Hawaii.

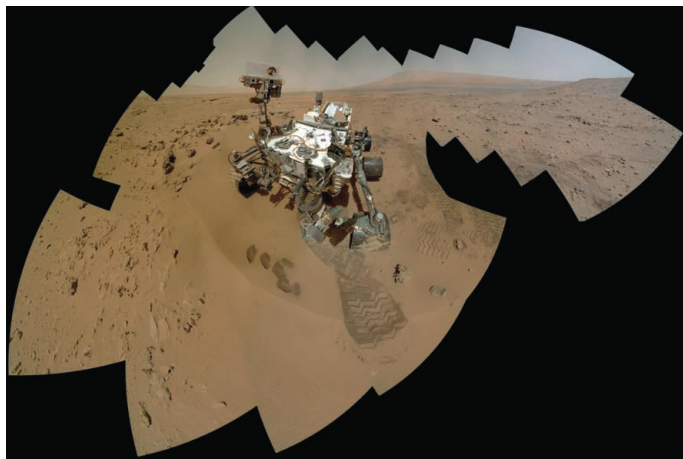
>> Read the full article at <http://dx.doi.org/10.1126/science.1238932>

Curiosity at Gale Crater, Mars: Characterization and Analysis of the Rocknest Sand Shadow

D. F. Blake,* R. V. Morris, G. Kocurek, S. M. Morrison, R. T. Downs, D. Bish, D. W. Ming, K. S. Edgett, D. Rubin, W. Goetz, M. B. Madsen, R. Sullivan, R. Gellert, I. Campbell, A. H. Treiman, S. M. McLennan, A. S. Yen, J. Grotzinger, D. T. Vaniman, S. J. Chipera, C. N. Achilles, E. B. Rampe, D. Sumner, P.-Y. Meslin, S. Maurice, O. Forni, O. Gasnault, M. Fisk, M. Schmidt, P. Mahaffy, L. A. Leshin, D. Glavin, A. Steele, C. Freissinet, R. Navarro-González, R. A. Yingst, L. C. Kah, N. Bridges, K. W. Lewis, T. F. Bristow, J. D. Farmer, J. A. Crisp, E. M. Stolper, D. J. Des Marais, P. Sarrazin, MSL Science Team†

*Corresponding author. E-mail: david.blake@nasa.gov
National Aeronautics and Space Administration (NASA) Ames Research Center, Moffett Field, CA 94035, USA.

†MSL Science Team authors and affiliations are listed in the supplementary materials. The list of author affiliations is available in the full article online.



The Rocknest aeolian deposit is similar to aeolian features analyzed by the Mars Exploration Rovers (MERs) Spirit and Opportunity. The fraction of sand <150 micrometers in size contains ~55% crystalline material consistent with a basaltic heritage and ~45% x-ray amorphous material. The amorphous component of Rocknest is iron-rich and silicon-poor and is the host of the volatiles (water, oxygen, sulfur dioxide, carbon dioxide, and chlorine) detected by the Sample Analysis at Mars instrument and of the fine-grained nanophase oxide component first described from basaltic soils analyzed by MERs. The similarity between soils and aeolian materials analyzed at Gusev crater, Meridiani Planum, and Gale crater implies locally sourced, globally similar basaltic materials or globally and regionally sourced basaltic components deposited locally at all three locations.

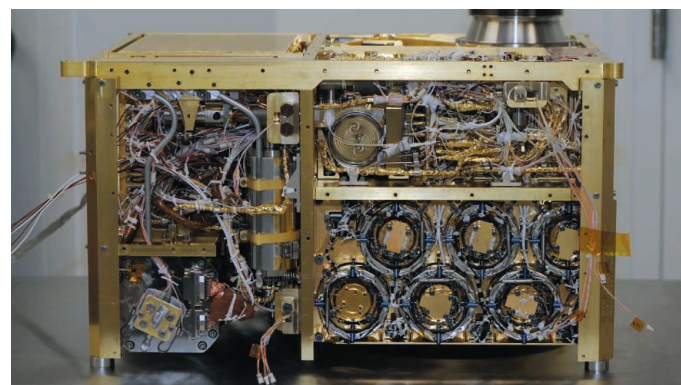
>> Read the full article at <http://dx.doi.org/10.1126/science.1238950>

Volatile, Isotope, and Organic Analysis of Martian Fines with the Mars Curiosity Rover

L. A. Leshin,* P. R. Mahaffy, C. R. Webster, M. Cabane, P. Coll, P. G. Conrad, P. D. Archer Jr., S. K. Atreya, A. E. Brunner, A. Buch, J. L. Eigenbrode, G. J. Flesch, H. B. Franz, C. Freissinet, D. P. Glavin, A. C. McAdam, K. E. Miller, D. W. Ming, R. V. Morris, R. Navarro-González, P. B. Niles, T. Owen, R. O. Pepin, S. Squyres, A. Steele, J. C. Stern, R. E. Summons, D. Y. Sumner, B. Sutter, C. Szopa, S. Teinturier, M. G. Trainer, J. J. Wray, J. P. Grotzinger, MSL Science Team†

*Corresponding author. E-mail: leshin@rpi.edu
Department of Earth and Environmental Sciences and School of Science, Rensselaer Polytechnic Institute, Troy, NY 12180, USA.

†MSL Science Team authors and affiliations are listed in the supplementary materials. The list of author affiliations is available in the full article online.



Samples from the Rocknest aeolian deposit were heated to ~835°C under helium flow and evolved gases analyzed by Curiosity's Sample Analysis at Mars instrument suite. H₂O, SO₂, CO₂, and O₂ were the major gases released. Water abundance (1.5 to 3 weight percent) and release temperature suggest that H₂O is bound within an amorphous component of the sample. Decomposition of fine-grained Fe or Mg carbonate is the likely source of much of the evolved CO₂. Evolved O₂ is coincident with the release of Cl, suggesting that oxygen is produced from thermal decomposition of an oxychloride compound. Elevated δD values are consistent with recent atmospheric exchange. Carbon isotopes indicate multiple carbon sources in the fines. Several simple organic compounds were detected, but they are not definitively martian in origin.

>> Read the full article at <http://dx.doi.org/10.1126/science.1238937>

References

1. J. P. Grotzinger *et al.*, Mars Science Laboratory mission and science investigation. *Space Sci. Rev.* **170**, 5 (2012).
2. R. M. E. Williams *et al.*, Martian fluvial conglomerates at Gale crater. *Science* **340**, 1068 (2013).



See all of *Science's* Curiosity coverage, including news, research, and multimedia, at www.sciencemag.org/extra/curiosity



The Petrochemistry of Jake_M: A Martian Mugearite

E. M. Stolper,^{1*} M. B. Baker,¹ M. E. Newcombe,¹ M. E. Schmidt,² A. H. Treiman,³ A. Cousin,^{4,5} M. D. Dyar,⁶ M. R. Fisk,⁷ R. Gellert,⁸ P. L. King,⁹ L. Leshin,¹⁰ S. Maurice,⁵ S. M. McLennan,¹¹ M. E. Minitti,¹² G. Perrett,⁸ S. Rowland,¹³ V. Sautter,¹⁴ R. C. Wiens,⁴ MSL Science Team†

“Jake_M,” the first rock analyzed by the Alpha Particle X-ray Spectrometer instrument on the Curiosity rover, differs substantially in chemical composition from other known martian igneous rocks: It is alkaline (>15% normative nepheline) and relatively fractionated. Jake_M is compositionally similar to terrestrial mugearites, a rock type typically found at ocean islands and continental rifts. By analogy with these comparable terrestrial rocks, Jake_M could have been produced by extensive fractional crystallization of a primary alkaline or transitional magma at elevated pressure, with or without elevated water contents. The discovery of Jake_M suggests that alkaline magmas may be more abundant on Mars than on Earth and that Curiosity could encounter even more fractionated alkaline rocks (for example, phonolites and trachytes).

Rock “Jake_M” (JM; named for Jet Propulsion Laboratory engineer Jake Matijevic) was the first sample imaged with the Mars Hand Lens Imager (MAHLI) and analyzed with the Alpha Particle X-ray Spectrometer (APXS) on the Mars Science Laboratory (MSL) (1, 2). Although the rock is an isolated fragment lacking field context (encountered ~282 m from the Bradbury landing site and analyzed on sols 46 and 47, where 1 sol is a martian day), its dark color and apparently fine-grained texture suggested, before analysis, that it was a relatively homogeneous (on a millimeter-to-centimeter scale) igneous rock and thus an appropriate sample with which to initiate the APXS analytical program and to analyze with ChemCam (3) using laser-induced breakdown spectroscopy (LIBS). Here, we report chemical analyses of JM and an interpretation of their meaning for its petrogenesis.

Results and Discussion

Petrography

Jake_M is roughly pyramidal in shape (~50 cm on each of its three base edges and ~50 cm tall)

(Fig. 1). The rock is dark gray and thinly coated by light-toned, reddish-brown dust. Its upper surfaces have rounded hollows that are probably due to wind erosion and <1- to 3-mm pits that could be vesicles. The lowest ~2 cm of the rock has smoother surfaces that may reflect primary layering or the effects of wind erosion. Near-vertical fractures (~10 cm long) project upward from the base. Feldspar microphenocrysts have been tentatively identified in MAHLI images (4), but individual mineral grains could not otherwise be distinguished in optical images, perhaps due to the dust cover and/or polish by wind. Compositional variations among the 14 individual

locations (see Fig. 1 and fig. S1) analyzed by LIBS using ChemCam show that the rock is heterogeneous on a length scale of ~0.5 mm. The heterogeneities observed by LIBS analyses suggest the presence of plagioclase (broadly consistent with oligoclase), Ca-rich pyroxene, olivine, and Fe-Ti-rich oxide(s) (3) (see also figs. S1 to S4).

Bulk Composition and Classification

The three APXS analyses (Table 1) were collected on two different spots; the listed uncertainties on the average [calculated after normalizing each analysis to 100 weight % (wt %), excluding SO₃, Cl, and trace elements] are due to variations between the three analyses that may partially reflect real differences between the two analyzed spots. The surface of JM was not brushed or abraded before analysis [in the supplementary materials, we compare the JM analyses to both unbrushed (i.e., “as is”) and physically abraded rock surfaces analyzed by the Mars Exploration Rovers (MERs)]. The CIPW norms (5) (Table 1) are based on the average JM composition and were calculated using molar Fe³⁺/(total Fe) ratios of 0 and 0.15. Although this range of Fe³⁺/(total Fe) ratios brackets the ratios expected in basaltic melts at the estimated oxygen fugacity (*f*_{O₂}) values of basaltic shergottites [e.g., (6)], recent modeling suggests that mantle melting at higher *f*_{O₂}s may have occurred early in the planet’s history (7). However, even for a Fe³⁺/(total Fe) value of 0.3, normative nepheline is still ~15 wt %.

Based on either its calculated norm or inspection of its major-element composition, JM has a basaltic composition, and it is probably an igneous rock (although we cannot tell whether it is from a lava flow, an intrusion, a pyroclastic flow, or a

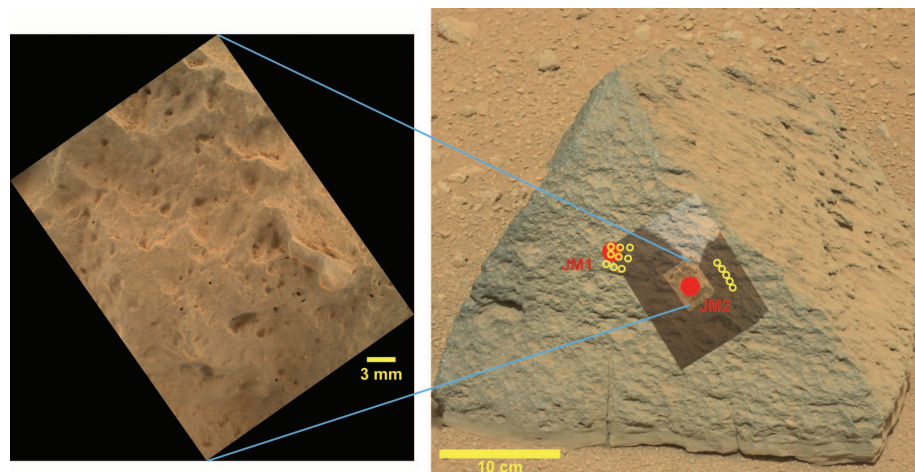


Fig. 1. Raw image of Jake_M taken by the left mast camera (mastcam) (identification number 0046ML0212000000E1) with overlain images from MAHLI at 26.9-, 6.9-, and 4.4-cm offsets from the front of the lens. The MAHLI projection on the left was taken at 4.4 cm (identification number 0047MH0011002000E1). Shadowing by the turret reduced the contrast in the inset MAHLI images, causing color differences with the mastcam image. The solid red circles labeled JM1 and JM2 indicate the locations of the two APXS spots (1.7-cm diameter). ChemCam raster spots are represented by yellow open circles; actual spot sizes are ~0.45 mm. [Credit: NASA/Jet Propulsion Laboratory–Caltech/Malin Space Science Systems]

¹Caltech, Pasadena, CA 91125, USA. ²Brock University, St. Catharines, Ontario L2T 3V8, Canada. ³Lunar and Planetary Institute, Houston, TX 77058, USA. ⁴Los Alamos National Laboratory, Los Alamos, NM 87545, USA. ⁵Institut de Recherches en Astrophysique et Planétologie, 31028 Toulouse, France. ⁶Mount Holyoke College, South Hadley, MA 01075, USA. ⁷Oregon State University, Corvallis, OR 97331, USA. ⁸University of Guelph, Guelph, Ontario N1G 2W1, Canada. ⁹Research School of Earth Sciences, Australian National University, Acton, ACT 0200, Australia. ¹⁰Rensselaer Polytechnic Institute, Troy, NY 12180, USA. ¹¹The State University of New York, Stony Brook, NY 11794, USA. ¹²Applied Physics Laboratory, The Johns Hopkins University, Baltimore, MD 20723, USA. ¹³University of Hawaii, Honolulu, HI 96822, USA. ¹⁴Laboratoire de Minéralogie et Cosmochimie du Muséum, 75005 Paris, France.

*Corresponding author. E-mail: ems@gps.caltech.edu

†MSL Science Team authors and affiliations are listed in the supplementary materials.

volcaniclastic sediment deposited after minimal fractionation or alteration of primary igneous materials). Moreover, with its ~16 to 17% normative nepheline (Table 1) and its position on an alkali-silica diagram (Fig. 2), JM is an alkaline rock [with an alkalinity index (8) higher than other known martian rocks]. JM is also evolved (likely due to crystal fractionation) relative to most other known martian igneous rocks (Fig. 3): It has a low MgO content (4.4 wt %), albitic normative plagioclase (oligoclase, ~An₁₅), a molar Mg/(Mg+Fe_{total}) ratio of ~0.43, ~40 parts per million (ppm) Ni, and ~270 ppm Cr (Ni and Cr values are from the two long-duration analyses listed in Table 1). Based on its MgO content, JM is more fractionated than most other martian rocks—of the analyses plotted in Fig. 3, only the basaltic shergottite Los Angeles (9), the rocks Wishstone and Champagne analyzed by the MERs (10), and the two estimated soil-free Pathfinder rock compositions (11, 12) have similar or lower MgO contents.

For terrestrial igneous rocks, chemical composition is generally not the sole criterion for classification. For JM, we have no other information and although it plots slightly above the nominal mugearite field on the alkali-silica diagram (this field is shown as the blue polygon in Fig. 2), the composition of the normative plagioclase (i.e., oligoclase; broadly consistent with the ChemCam results), the substantial normative nepheline and orthoclase, and the fact that it overlaps compositionally with many terrestrial

rocks that have historically been called mugearites (Fig. 2B and fig. S5) lead us to classify JM as a mugearite (13). Mugearites are well-defined and widely distributed, though relatively uncommon, intermediate (i.e., fractionated) members of the terrestrial alkali-olivine basalt, hawaiite, mugearite, benmoreite, trachyte-phonolite magma series found in locations such as ocean islands and continental rifts (14–16). They generally contain normative nepheline, but nepheline as a phenocryst phase is relatively rare (14), so the absence of a nepheline signature in the ChemCam results is not inconsistent with JM’s normative composition. Although JM actually plots in the nominal phonotephrite field in Fig. 2, in other respects the compositional comparison of JM to terrestrial rocks that have been called phonotephrites is no better (and arguably worse) than to rocks called mugearites (figs. S5 and S6).

Comparison to Other Martian Igneous Rocks

Although there is overlap in some oxide concentrations, taken as a whole, the JM composition is distinct from all other known martian igneous rocks (Figs. 2 and 3). In particular, compared with JM’s Na₂O and K₂O contents of ~7 and ~2.1 wt %, respectively (Table 1), all martian meteorites and martian igneous rocks analyzed by Pathfinder and the MERs are considerably lower in sodium and potassium: The highest previously analyzed Na₂O contents are only ~4 to 5 wt % [Backstay, Humboldt Peak, Northwest Africa

(NWA) 7034 meteorite, Wishstone, Champagne, and one of the estimated soil-free Pathfinder compositions) (Fig. 3F), and the highest K₂O contents of relatively unaltered martian rocks (17) are only ~1 wt % (Backstay, Humboldt Peak, Madeline English, and the soil-free Pathfinder compositions) (Fig. 3G). However, there is evidence from the nakhlite meteorites of K-rich martian liquids: (i) the presence of K-rich kaersutite in melt inclusions (18), (ii) highly fractionated glassy mesostasis in the nakhlites (19), and (iii) K-rich bulk melt-inclusion compositions (20). Most martian meteorites and analyzed martian igneous rocks have higher MgO and FeO* contents and lower Al₂O₃ contents than JM (Fig. 3, C and D) [see also (21)]. Although there are exceptions for individual elements (e.g., the soil-free Pathfinder compositions, Wishstone, Champagne, and Los Angeles for MgO; Backstay, Wishstone, Champagne, and NWA 7034 for FeO*; and Wishstone and Champagne for Al₂O₃), no known martian rock overlaps JM in all three of these elements. The Ni (22 to 59 ppm) and Cr (~270 ppm) contents of JM are among the lowest values for an unbrushed rock surface found on Mars to date; moreover, because martian dust is typically enriched in Ni by ~10 times the JM values (22), JM probably contains even lower Ni than is suggested by the APXS analyses.

Although some Gusev samples are alkaline [i.e., they plot above the alkaline-subalkaline boundary curve in Fig. 2 (23) and have normative

Table 1. Composition and CIPW norms of Jake_M. 1 and 2 after JM indicate the two locations analyzed on the rock (see Fig. 1); 2n indicates the nighttime analysis on spot 2. Values in parentheses for JM1, JM2, and JM2n are assessments of 2σ uncertainty based on counting statistics and data reduction in terms of the least number of units cited [i.e., 50.7(6) = 50.7 ± 0.6]; for further details see (2). n.d., not detected; N/A, not applicable. The average represents the unweighted mean of the three compositions, each normalized to 100% excluding SO₃, Cl, and trace elements; values in parentheses are the standard deviations. Norm, normative minerals in weight %. The column labeled “0 Fe³⁺” shows the calculated CIPW norm, as-

suming that all Fe in the average bulk composition is Fe²⁺; the column labeled “0.15 Fe³⁺” shows the calculated CIPW norm, assuming Fe³⁺/(total Fe) = 0.15. Normative constituents: Pl, plagioclase (sum of normative anorthite and albite); Or, orthoclase; Ne, nepheline; Cpx, sum of normative diopside and hedenbergite; Ol, sum of normative forsterite and fayalite; Ilm, ilmenite; Mt, magnetite; Ap, apatite; Chr, chromite; %An, 100 × Ca/(Ca + Na)_{molar} in the normative plagioclase; Mg# ol and Mg# cpx = 100 × Mg/(Mg + Fe)_{molar} in normative olivine and high-Ca pyroxene, respectively; (Mg#)^{ol} = 100 × Mg/(Mg + Fe)_{molar} of the liquidus olivine calculated using an olivine-liquid K_{D,Fe²⁺-Mg} [(FeO/MgO)^{ol}/(FeO/MgO)^{liquid}] of 0.34 (80, 81).

Weight %	JM1	JM2	JM2n	Average	Norm	0 Fe ³⁺	0.15 Fe ³⁺
SiO ₂	50.7(6)	49.3(9)	48.9(5)	51.6(9)	Pl	32.3	34.4
TiO ₂	0.50(3)	0.65(6)	0.73(3)	0.65(12)	Or	12.5	12.5
Al ₂ O ₃	16.1(5)	14.6(7)	14.6(2)	15.7(9)	Ne	17.4	16.2
Cr ₂ O ₃	0.03(1)	0.09(3)	0.04(1)	0.04(1)	Cpx	20.0	19.8
FeO	9.44(7)	10.61(11)	10.94(9)	10.8(8)	Ol	14.9	11.6
MnO	0.14(1)	0.17(2)	0.21(1)	0.18(4)	Ilm	1.2	1.2
MgO	3.6(4)	4.6(7)	4.60(12)	4.4(6)	Mt	N/A	2.6
CaO	6.09(7)	6.54(11)	6.78(8)	6.7(4)	Ap	1.6	1.6
Na ₂ O	7.1(3)	6.6(5)	5.59(14)	7.0(3)	Chr	0.06	0.06
K ₂ O	2.22(4)	2.01(6)	1.89(3)	2.12(17)	%An	15.2	14.2
P ₂ O ₅	0.50(7)	0.60(12)	0.85(4)	0.68(19)	Mg# ol	43.0	49.7
SO ₃	2.46(9)	3.05(16)	2.81(8)	N/A	Mg# cpx	43.0	49.7
Cl	0.88(3)	1.03(5)	0.95(3)	N/A	(Mg#) ^{ol}	68.0	71.6
Total	99.80	99.80	99.90	N/A	N/A	N/A	N/A
Ni (ppm)	22(17)	n.d.	59(17)	N/A	N/A	N/A	N/A
Zn (ppm)	216(13)	341(25)	318(15)	N/A	N/A	N/A	N/A
Br (ppm)	88(8)	94(11)	107(7)	N/A	N/A	N/A	N/A
Temp	−3°C	−2°C	−55°C	N/A	N/A	N/A	N/A
Duration	30 min	12 min	30 min	N/A	N/A	N/A	N/A

nepheline; e.g., Humboldt Peak] or transitional (i.e., they plot near the boundary curve and have only small amounts of either normative nepheline or hypersthene; e.g., Backstay, NWA 7034), no relatively unaltered samples are as alkali-rich relative to the alkaline-subalkaline boundary curve in Fig. 2 or as rich in normative nepheline as JM. Note that despite their positions in Fig. 2, Wishstone and Champagne are not nepheline-normative, due to their extremely high bulk P_2O_5 contents of 5.2 to 5.3 wt %. Only if P_2O_5 were ~ 1 wt %, a value more typical of Gusev crater rocks, would these rocks be as strongly nepheline-normative as their positions on Fig. 2 might suggest. [See also (24) for a discussion of how changing the normative phosphate-bearing mineral from apatite to Ca-merrillite affects the proportions of the other normative components.]

Comparison to Terrestrial Compositions

As shown by a comparison between JM and lavas from Tenerife (one of the Canary Islands), there is an excellent correspondence between JM and fractionated alkaline basaltic rocks on Earth (Fig. 3 and figs. S5 to S8). JM lies on or near the oxide-MgO trends for Tenerife for all oxides except TiO_2 . As is the case for JM, when compared with Tenerife lavas with the same MgO content, nearly all of the martian rocks plotted in Fig. 3 have substantially lower TiO_2 contents than the Tenerife lavas, and this low TiO_2 appears to be a characteristic of martian rocks generally. Nevertheless, even the TiO_2 content of JM is not outside

the range of fractionated terrestrial alkaline igneous rock compositions (fig. S7A), and both JM and nonalkaline martian rocks overlap with terrestrial tholeiites in TiO_2 -MgO space (fig. S7B). Although JM is slightly elevated in total alkalis relative to the Tenerife trend (Fig. 2) and at the upper end of the field defined by mugearite lavas (reflecting JM's high Na_2O content) (Fig. 3F and fig. S5F), terrestrial alkaline suites span a wide range of total alkali contents at a given silica (or MgO) content, with some being lower than that of JM [e.g., St. Helena (25)] and others being higher [e.g., Tristan da Cunha (25)].

The chemical similarity between JM and terrestrial igneous rocks is surprising given that the chemical compositions of SNC (Shergotty, Nakhla, and Chassigny) meteorites and of igneous rocks analyzed using APXS on the surface of Mars (after correction for or removal by brushing or abrasion of surface-correlated components such as dust) differ systematically, in many respects, from those of terrestrial igneous rocks [e.g., (26, 27); see also (21)]. These distinctions remain even when martian meteorites are compared with Fe-rich terrestrial lavas (28). However, even JM's Fe/Mn ratio is within the range of comparable terrestrial igneous rocks [fig. S8; terrestrial and martian bulk rock and olivine and pyroxene Fe/Mn ratios have historically been considered diagnostic of each planet (29–32)]. Overall, if JM had been found on Earth, we would be hard pressed to tell from its whole-rock chemical composition that it is martian. In the discussion below, we use the fact that JM

plots essentially on the alkaline rock series from Tenerife (Figs. 2 and 3) as an aid to understanding one possible model for its petrogenesis.

Based on the differences in S and Cl contents from undisturbed versus physically abraded martian rock surfaces, it is likely that much of the S and Cl in the APXS analysis of JM reflects a surficial component such as dust [after abrasion, SO_3 and Cl contents of Gusev crater rocks and outcrops generally drop by ~ 40 to 90% (10)]. However, there are h  yine-bearing terrestrial lavas, historically called “tahitites” (33), with major-element compositions that are broadly similar to JM and with elevated S and Cl contents. Analyses of such h  yine-bearing lavas (containing 50 to 58 wt % SiO_2 on a volatile-free basis) from the Georoc database (25) have 0.6 to 2.4 wt % SO_3 and up to 0.8 wt % Cl [and some phonolitic lavas from Tenerife contain h  yine; e.g., (34)]. Thus, although it cannot be quantified at this time, it is possible that non-negligible amounts of the S and Cl in the JM analysis are indigenous to the magma from which JM formed rather than a secondary, surface-correlated feature.

Petrogenesis of JM

Hypotheses for the origins of igneous rocks rarely rely on isolated chemical compositions but are constrained by field relations, petrography, and the compositional trends defined by related rocks. We lack these data for JM, but we are able to say with some confidence what its compositional features would signify if it formed by processes

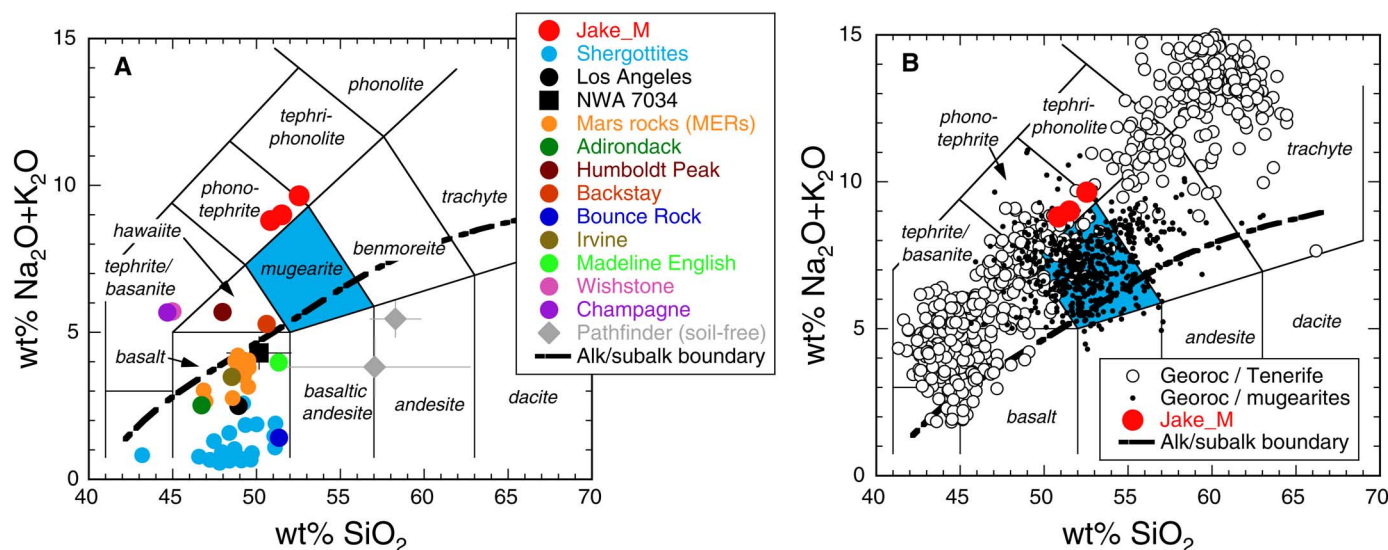


Fig. 2. Alkali-silica diagram. Compositional boundaries and rock names are from (72); the mugearite field is shown in blue. The dashed curve shows the alkaline-subalkaline boundary curve from Irvine and Baragar (23). (A) Colored symbols (see the key) show the three JM analyses (Table 1), normalized to 100 wt % without SO_3 , Cl, and trace elements; basaltic martian meteorites [the shergottite “Los Angeles” (9, 73) and the basaltic breccia NWA 7034 (74) are shown as distinct symbols]; martian rocks analyzed by the MERs (10, 75–77) and interpreted as igneous (including volcanoclastics); and the two soil-free Pathfinder compositions calculated by W  nke *et al.* (11) and Foley *et al.* (12). Errors bars associated with the NWA 7034 and Pathfinder compositions

reflect either 1σ uncertainties (NWA 7034) or the projection methods used to calculate a soil-free composition (Pathfinder). Note that NWA 7034 may be a polymict breccia (78, 79). Larger filled colored circles labeled “Adirondack” through “Champagne” in the key denote specific Mars surface rocks analyzed by the MERs. (B) Comparison of the three JM analyses (Table 1) with lavas from Tenerife in the Canary Islands (25) and with terrestrial lavas that have been called mugearites, including some from Tenerife (25). Only Georoc (25) analyses with oxide sums between 97 and 102.5 wt % are plotted, and all have been normalized to 100 wt % on a volatile-free (including sulfur and chlorine) basis.

similar to those that have produced comparable terrestrial rocks. Although an infinite number of petrogenetic models could be constructed to account for a single rock composition such as JM, we emphasize again the strong compositional correspondence between JM and terrestrial mugearites (including JM's position close to the liquid line of descent of Tenerife magmas). This correspondence provides a plausible context for interpreting the composition of JM and is at least permissive that the petrogenetic processes responsible for the compositional trends observed in these terrestrial lavas could be applicable to the evolution of JM.

Evolved terrestrial alkaline rocks, including mugearites, are generally produced by extensive crystal fractionation of alkaline or transitional magmas. Whereas in some cases this fractionation appears to occur in the upper mantle, based on the presence of peridotite xenoliths in some mugearites and related rocks (35–37), it more commonly occurs in crustal magma chambers or at even shallower depths within a volcanic edifice [e.g., (15, 16, 38)]. With this in mind, we used MELTS software (39, 40) to simulate fractional crystallization of a primitive Tenerife melt composition over a range of pressures (1 to 6000 bars), water concentrations (0 to 3 wt %), and oxygen fugacities [quartz-fayalite-magnetite (QFM) – 1 to QFM + 2].

A crucial constraint on the fractionation required to explain the trend of Tenerife magmas is the monotonic increase in the Al_2O_3 contents of the observed rocks with decreasing MgO content (at least down to 2 wt % MgO). As shown by MELTS calculations (Fig. 4), this monotonic change all the way down to 2 wt % MgO cannot be produced by fractionation from a dry primitive basanite at 1 bar: Under these conditions, plagioclase saturation is reached at ~7.8 wt % MgO, long before sufficient fractionation has occurred to produce residual liquids with MgO contents in the 2% range. As a result, residual liquids at 1 bar with MgO contents like those of JM (4 to 5 wt %) contain only 14 to 15 wt % Al_2O_3 (i.e., less than the ~15 to 19 wt % Al_2O_3 in JM and terrestrial mugearites) (fig. S5). To produce residual melts with monotonically increasing Al_2O_3 contents at these MgO contents, plagioclase crystallization must be suppressed. It is well known that elevated water contents and elevated total pressure individually or together can suppress plagioclase crystallization (41–44). MELTS calculations confirm this: Starting with the primitive basanite at 4 kbar dry, the MELTS calculations predict that plagioclase saturation is delayed relative to 1 bar crystallization, being reached only at liquid MgO contents of ~4.8 wt % (Fig. 4). In contrast, and as expected, clinopyroxene saturates earlier in the fractionation sequence relative to the calculated trend at 1 bar (Fig. 4). With the addition of 1 wt % H_2O to the parental basanite at 4 kbar, plagioclase crystallization is even further suppressed: Figure 4 shows that the model fractionation sequence reaches plagioclase saturation only at ~2 wt % MgO and that clinopyroxene appearance is also

somewhat delayed relative to the 4-kbar anhydrous calculation. Note that the points along the model liquid lines of descent that mark the appearance of Fe-rich spinel are only slightly affected over the ranges in pressure and water content investigated here (Fig. 4 and fig. S11). These calculations were all done at an f_{O_2} fixed relative to the QFM buffer [i.e., at QFM + 1, an f_{O_2} consistent with estimates

from Fe-Ti oxides in Tenerife volcanics (38)]; under more oxidizing or reducing conditions, Fe-rich spinel would appear earlier or later in the calculated liquid line of descent.

Although the 4-kbar dry simulation of the Tenerife parental basanite suppresses plagioclase crystallization sufficiently to account for the high Al_2O_3 contents of JM and of rocks from

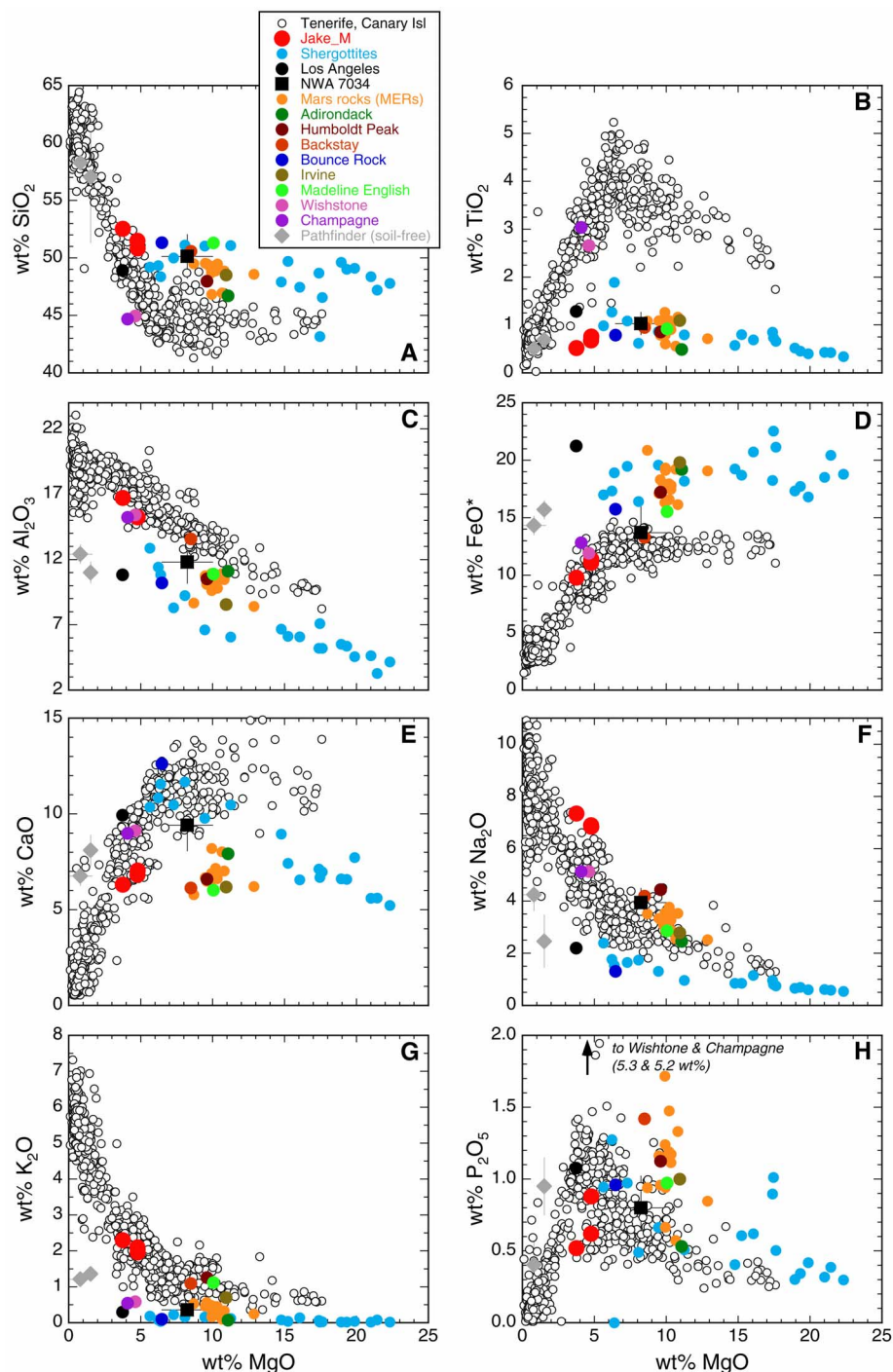


Fig. 3. Oxide-MgO variation diagrams (weight %) comparing Tenerife lavas, the three Jake_M compositions (Table 1), and various martian igneous rock compositions (see caption to Fig. 2 for references and filters applied to the Tenerife lava compositions). (A) SiO_2 -MgO; (B) TiO_2 -MgO; (C) Al_2O_3 -MgO; (D) FeO^* -MgO, where FeO^* denotes all Fe as FeO; (E) CaO -MgO; (F) Na_2O -MgO; (G) K_2O -MgO; and (H) P_2O_5 -MgO. Error bars are as in Fig. 2.

Tenerife with 4 to 5 wt % MgO, the simulated fractionation trend provides a poor fit to the more evolved lavas from Tenerife (Fig. 4), which would require even further suppression of plagioclase crystallization to account for their even higher Al_2O_3 contents. In contrast, the 4 kbar simulation with 1 wt % H_2O in the parent magma reproduces the observed trend in Al_2O_3 all the way down to ~2 wt % MgO (Fig. 4), reproduces reasonably well the trends of all of the other oxides (fig. S11), and matches the water contents measured in melt inclusions from Tenerife lavas with phonolitic compositions (45). The simulation at 4 kbar with 1 wt % H_2O in the parental basanite magma, which reaches the MgO content of JM after ~57% crystallization and with ~2.3 wt % H_2O in the JM-like residual melt, provides the best fit to the overall Tenerife trend (see fig. S12, which illustrates the degree to which the calculated liquid lines of descent reproduce the compositional trend of the lavas as pressure and initial water content vary). Similar calculations by Beier *et al.* (46) using lavas from Sete Cidades volcano, Sao Miguel (in the Azores) produced comparable results, requiring 0.5 wt % H_2O in the parent liquid and fractionation at 5 kbar to reproduce the overall observed liquid line of descent.

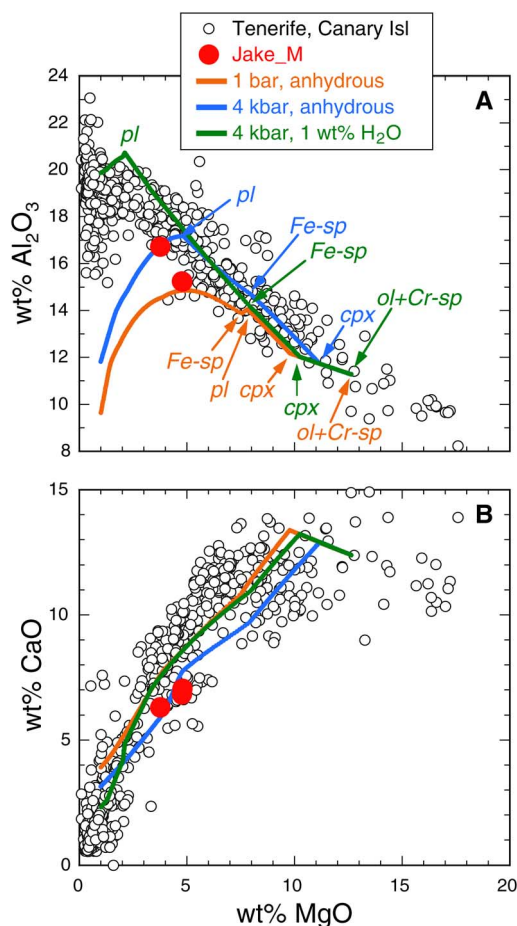
The point of these simulations and their comparisons to JM and to the overall Tenerife

liquid line of descent (Fig. 4 and fig. S11) is not whether a precise match can be achieved. As good as they are, MELTS calculations are no substitute for experiments in determining a fractionation path and its sensitivity to pressure, water content, other volatiles, and oxygen fugacity [e.g., the calculated best-fit liquid line of descent does not include amphibole and yet, amphibole is present in the more fractionated Tenerife lavas (38)]. Moreover, it is unreasonable to suppose that JM's bulk composition represents exactly a liquid composition or that the parent magma would be identical in all respects to one from Tenerife (as pointed out above, the Na_2O and TiO_2 contents of JM and the Tenerife trend do not match perfectly). The point of the comparison is simply that the overall trend of the Tenerife liquid line of descent is captured reasonably well only if plagioclase crystallization is suppressed relative to low-pressure, dry conditions and that several kilobars of pressure (corresponding to up to a few tens of kilometers depth within Mars) and water contents in the parent magma on the order of 1 wt % H_2O do this successfully. If the pressure were less than ~1 kbar, the fits worsen because, under these conditions, not enough water is able to dissolve in the melt to suppress plagioclase crystallization sufficiently to reproduce the monotonic enrichment with fractionation observed in Al_2O_3

among the most highly evolved rocks from Tenerife. Nevertheless, as stated above, the 4-kbar anhydrous trend provides a reasonable fit to the JM compositions, and thus we cannot say with any confidence that the fractionation of JM requires ~1 wt % H_2O in the parental magma. Although the model 1-bar fractionation trend at 4 to 5 wt % MgO is low in Al_2O_3 relative to JM, if we allowed for moderate plagioclase accumulation in JM or increased uncertainties in JM's stated composition, even fractionation under these conditions could not be ruled out. However, we can say with reasonable certainty that terrestrial magmas that are compositionally similar to JM require fractionation at both elevated pressure and water content. One way to resolve this for JM would be if more evolved alkaline lavas are discovered on Mars and whether these, like comparable terrestrial magmas, have even higher Al_2O_3 contents than JM. If so, this would strengthen the requirement for a moderate-pressure, hydrous liquid line of descent to explain JM because it would be difficult to match such elevated Al_2O_3 contents at low pressure or without dissolved water. Although they are not definitive, the pits on the surface of JM (Fig. 1) may be wind-eroded vesicles, which would be consistent with hydrous fractionation. Likewise, the inferred water content of JM (~2 wt %, if we accept the analogy with Tenerife magmas) is also consistent with previous efforts to constrain the petrogenesis of martian magmas, which have concluded that they contained up to several weight % dissolved H_2O (47–49). Measurements of water in amphiboles in Chassigny (50) also suggest that the mantle source region of Chassigny may have been relatively wet. In contrast, however, Filiberto and Treiman (51) have argued that magmas parental to the martian meteorites were chlorine-rich and water-poor; that is, <0.3 wt % H_2O . Although extensive work has been done on the partitioning of Cl between silicate melts and H_2O -rich fluids [e.g., (52, 53)], it is not clear from available experimental data [e.g., (54)] whether Cl suppresses plagioclase crystallization to a similar degree as H_2O .

To explore whether any known martian igneous rocks could represent acceptable parent liquids for JM, we also performed MELTS calculations on Backstay, Humboldt Peak, and NWA 7034. In these cases, because there is no suite of lavas to constrain the liquid line of descent as in the case of the Tenerife calculations, we used MELTS simulations only to determine whether parent liquids corresponding to these known martian igneous rocks could fractionate to produce a residual liquid corresponding to JM and, if so, what conditions would be required. None of the martian rock compositions have high enough alkali contents to produce a close match to JM under any conditions (figs. S13 to S24). However, if we arbitrarily increased the alkali contents by amounts such that on fractionation the modeled alkali contents of the fractionated liquids matched those of JM at an MgO content of ~4 to 5 wt %, the alkali-enriched Backstay composition could produce a reasonable

Fig. 4. Oxide-MgO variation diagrams (weight %) showing Tenerife lavas, the three Jake_M compositions, and three MELTS calculations. (A) Al_2O_3 -MgO and (B) CaO-MgO; MELTS fractional crystallization calculations (colored curves) are described in the text (1 bar, anhydrous; 4 kbar, anhydrous; 4 kbar, 1 wt % water in the parental liquid composition; all three calculations were done at $f_{\text{O}_2} = \text{QFM} + 1$). Phase abbreviations: pl, plagioclase; Fe-sp, magnetite-rich spinel; cpx, clinopyroxene; ol, olivine; Cr-sp, chromite-rich spinel. Arrows point to the appearance of phases along the MELTS-modeled liquid lines of descent. Compositions of the Tenerife lavas are from (25) (see caption to Fig. 2); starting composition for the MELTS modeling is the average of Tenerife lavas with 12 to 13.5 wt % MgO and is reported in the supplementary materials, along with further details of the MELTS calculations.



approximation of JM after a few tens of percent crystal fractionation (although we note that the required arbitrary increases in alkalis are not trivial).

Origins of Alkaline Magmas on Mars

We have no constraints on conditions required on Mars to produce the parental alkaline or transitional liquids from which JM is presumed to have evolved by extensive crystal fractionation. On Earth, such parental magmas have been attributed to a variety of conditions and processes, including melting of lherzolite + CO₂ ± H₂O at elevated pressures [e.g., (55–57)], melting of metasomatized lithospheric mantle [e.g., (58–60)], and melting of pyroxenites and amphibolites [e.g., (60–62)]. Models for the origin of previously described alkaline and transitional martian magmas have called on melting of a more alkali-rich mantle source [relative to that of the shergottites (63)] and/or hydrous fractional crystallization of transitional magmas at pressures of a few kilobars (64).

Ratios of moderately volatile alkalis to refractory lithophile elements in martian rocks have been used to infer that the primitive martian mantle was richer in Na and K than the terrestrial mantle by as much as a factor of 2 [e.g., (65–69)]. On this basis alone, although few alkaline martian rocks have been documented thus far, it would not be surprising if alkaline magmas derived from relatively alkali-rich sources (either primitive martian mantle or mantle that has been metasomatized by low-degree melts of relatively primitive mantle) were more common on Mars than they are on Earth [on Earth, alkaline lavas are rare from a planetary perspective, representing an estimated <1 volume % of terrestrial igneous rocks; e.g., (70)]. Note that based on trace-element and radiogenic-isotope ratios, the average sources of most shergottite meteorites are inferred to have been depleted and, in some cases, highly depleted (i.e., melts have been extracted from these source regions before the melting events that produced the shergottites). This depletion of their sources could explain the low alkali (and alumina) contents that are characteristic of the shergottites. If the liquids extracted during these earlier depletion events were enriched in alkalis (i.e., because they formed as partial melts of relatively primitive martian mantle) and were emplaced into the crust and lithospheric mantle, they could have enriched and metasomatized portions of the martian mantle. Melting of such enriched sources might then have produced the magmas parental to alkaline rocks such as JM. The overall K-rich nature of rocks analyzed by the MSL mission thus far (71) could reflect the presence of such an enriched region in the mantle underlying Gale crater.

References and Notes

1. J. P. Grotzinger *et al.*, Mars Science Laboratory: First 100 sols of geologic and geochemical exploration from Bradbury Landing to Glenelg. *Lunar Planet. Sci. Conf. 44*, abstract 1259 (2013).
2. R. Gellert *et al.*, Initial MSL APXS activities and observations at Gale Crater, Mars. *Lunar Planet. Sci. Conf. 44*, abstract 1432 (2013).
3. A. Cousin *et al.*, ChemCam analysis of Jake Matijevic, Gale Crater. *Lunar Planet. Sci. Conf. 44*, abstract 1409 (2013).
4. M. E. Minitti *et al.*, Mars Hand Lens Imager (MAHLI) observations of rocks at Curiosity's field site. *Lunar Planet. Sci. Conf. 44*, abstract 2186 (2013).
5. W. Cross, J. P. Iddings, L. V. Pirsson, H. S. Washington, *Quantitative Classification of Igneous Rocks* (Univ. of Chicago Press, Chicago, 1903).
6. M. Wadhwa, Redox conditions on small bodies, the Moon and Mars. *Rev. Mineral. Geochem.* **68**, 493–510 (2008). doi: [10.2138/rmg.2008.68.17](https://doi.org/10.2138/rmg.2008.68.17)
7. J. Tuff, J. Wade, B. J. Wood, Volcanism on Mars controlled by early oxidation of the upper mantle. *Nature* **498**, 342–345 (2013). doi: [10.1038/nature12225](https://doi.org/10.1038/nature12225); PMID: [23783628](https://pubmed.ncbi.nlm.nih.gov/23783628/)
8. J. M. Rhodes, Geochemical stratigraphy of lava flows sampled by the Hawaiian Scientific Drilling Project. *J. Geophys. Res.* **101**, 11729–11746 (1996). doi: [10.1029/95JB03704](https://doi.org/10.1029/95JB03704)
9. C. Meyer, The Martian Meteorite Compendium (2012); <http://curator.jsc.nasa.gov/antmet/mmc/index.cfm>.
10. R. Gellert *et al.*, Alpha Particle X-ray Spectrometer (APXS): Results from Gusev crater and calibration report. *J. Geophys. Res.* **111**, E02S05 (2006). doi: [10.1029/2005JE002555](https://doi.org/10.1029/2005JE002555)
11. H. Wänke, J. Brückner, G. Dreibus, R. Rieder, I. Ryabchikov, Chemical composition of rocks and soils at the Pathfinder site. *Space Sci. Rev.* **96**, 317–330 (2001). doi: [10.1023/A:1011961725645](https://doi.org/10.1023/A:1011961725645)
12. C. N. Foley, T. E. Economou, R. N. Clayton, W. Dietrich, Calibration of the Mars Pathfinder Alpha Proton X-ray Spectrometer. *J. Geophys. Res.* **108**, 8095 (2003). doi: [10.1029/2002JE002018](https://doi.org/10.1029/2002JE002018)
13. A. Harker, *The Tertiary Igneous Rocks of Skye. Memoirs of the Geological Survey of the United Kingdom* (James Hedderwick and Sons, Glasgow, 1904).
14. I. D. Muir, C. E. Tilley, Mugearites and their place in alkali igneous rock series. *J. Geol.* **69**, 186–203 (1961).
15. I. S. E. Carmichael, F. J. Turner, J. Verhoogen, *Igneous Petrology* (McGraw-Hill, New York, 1974).
16. M. Wilson, *Igneous Petrogenesis* (Unwin Hyman, London, 1989).
17. R. V. Morris *et al.*, Iron mineralogy and aqueous alteration from Husband Hill through Home Plate at Gusev crater, Mars: Results from the Mössbauer instrument on the Spirit Mars Exploration Rover. *J. Geophys. Res.* **113**, E12S42 (2008). doi: [10.1029/2008JE003201](https://doi.org/10.1029/2008JE003201)
18. V. Sautter, A. Jambon, O. Boudouma, Cl-amphibole in the nakhlite MIL 03346: Evidence for sediment contamination in a martian meteorite. *Earth Planet. Sci. Lett.* **252**, 45–55 (2006). doi: [10.1016/j.epsl.2006.09.024](https://doi.org/10.1016/j.epsl.2006.09.024)
19. J. M. D. Day, L. A. Taylor, C. Floss, H. Y. McSweeney Jr., Petrology and chemistry of ML 03346 and its significance in understanding the petrogenesis of nakhlites on Mars. *Meteorit. Planet. Sci.* **41**, 581–606 (2006). doi: [10.1111/j.1945-5100.2006.tb00484.x](https://doi.org/10.1111/j.1945-5100.2006.tb00484.x)
20. A. H. Treiman, The parent magma of the Nakhlite (SNC) meteorite, inferred from magmatic inclusions. *Geochim. Cosmochim. Acta* **57**, 4753–4767 (1993). doi: [10.1016/0016-7037\(93\)90198-6](https://doi.org/10.1016/0016-7037(93)90198-6)
21. H. Y. McSweeney Jr., G. J. Taylor, M. B. Wyatt, Elemental composition of the martian crust. *Science* **324**, 736–739 (2009). doi: [10.1126/science.1165871](https://doi.org/10.1126/science.1165871); PMID: [19423810](https://pubmed.ncbi.nlm.nih.gov/19423810/)
22. A. S. Yen *et al.*, Nickel on Mars: Constraints on meteoritic material at the surface. *J. Geophys. Res.* **111**, E12S11 (2006). doi: [10.1029/2006JE002797](https://doi.org/10.1029/2006JE002797)
23. T. N. Irvine, W. R. A. Baragar, A guide to the chemical classification of the common volcanic rocks. *Can. J. Earth Sci.* **8**, 523–548 (1971). doi: [10.1139/e71-055](https://doi.org/10.1139/e71-055)
24. T. Usui, H. Y. McSweeney Jr., B. C. Clark III, Petrogenesis of high-phosphorus Wishstone Class rocks in Gusev crater, Mars. *J. Geophys. Res.* **113**, E12S44 (2008). doi: [10.1029/2008JE003225](https://doi.org/10.1029/2008JE003225)
25. Georoc database; <http://georoc.mpch-mainz.gwdg.de/georoc/>.
26. H. Y. McSweeney Jr., What have we learned about Mars from SNC meteorites. *Meteoritics* **29**, 757–779 (1994). doi: [10.1111/j.1945-5100.1994.tb01092.x](https://doi.org/10.1111/j.1945-5100.1994.tb01092.x)
27. A. Ruzicka, G. A. Snyder, L. A. Taylor, Comparative geochemistry of basalts from the Moon, Earth, HED asteroid, and Mars: Implications for the origin of the Moon. *Geochim. Cosmochim. Acta* **65**, 979–997 (2001). doi: [10.1016/S0016-7037\(00\)00599-8](https://doi.org/10.1016/S0016-7037(00)00599-8)
28. J. Filiberto, Similarities between the shergottites and terrestrial ferropicrites. *Icarus* **197**, 52–59 (2008). doi: [10.1016/j.icarus.2008.04.016](https://doi.org/10.1016/j.icarus.2008.04.016)
29. E. Stolper, H. Y. McSweeney Jr., J. F. Hays, A petrogenetic model of the relationships among achondritic meteorites. *Geochim. Cosmochim. Acta* **43**, 589–602 (1979). doi: [10.1016/0016-7037\(79\)90167-4](https://doi.org/10.1016/0016-7037(79)90167-4)
30. H. Wänke, G. Dreibus, Chemical composition and accretion history of terrestrial planets. *Philos. Trans. R. Soc. London Ser. A* **325**, 545–557 (1988). doi: [10.1098/rsta.1988.0067](https://doi.org/10.1098/rsta.1988.0067)
31. J. Karner, J. J. Papike, C. K. Shearer, Olivine from planetary basalts: Chemical signatures that indicate planetary parentage and those that record igneous setting and process. *Am. Mineral.* **88**, 806–816 (2003).
32. J. Karner, J. J. Papike, C. K. Shearer, Comparative planetary mineralogy: Pyroxene major- and minor-element chemistry and partitioning of vanadium between pyroxene and melt in planetary basalts. *Am. Mineral.* **91**, 1574–1582 (2006). doi: [10.2138/am.2006.2103](https://doi.org/10.2138/am.2006.2103)
33. A. Lacroix, Les lavas à hâtiyne d'Auvergne et leurs enclaves homoeogènes. *Comptes Rendus* **CLXIV**, 581–587 (1917).
34. J. Andújar, F. Costa, J. Martí, J. A. Wolff, M. R. Carroll, Experimental constraints on pre-eruptive conditions of phonolitic magma from caldera-forming El Abrego eruption, Tenerife (Canary Islands). *Chem. Geol.* **257**, 173–191 (2008). doi: [10.1016/j.chemgeo.2008.08.012](https://doi.org/10.1016/j.chemgeo.2008.08.012)
35. J. F. G. Wilkinson, R. A. Binns, Hawaiite of high pressure origin from northeastern New South Wales. *Nature* **222**, 553–555 (1969). doi: [10.1038/222553a0](https://doi.org/10.1038/222553a0)
36. A. J. Stolz, Garnet websterites and associated ultramafic inclusions from a nepheline mugearite in the Walcha area, New South Wales, Australia. *Mineral. Mag.* **48**, 167–179 (1984). doi: [10.1180/minmag.1984.048.347.02](https://doi.org/10.1180/minmag.1984.048.347.02)
37. D. H. Green, A. D. Edgar, P. Beasley, E. Kiss, N. G. Ware, Upper mantle source for some hawaiites, mugearites and benmoreites. *Contrib. Mineral. Petrol.* **48**, 33–43 (1974). doi: [10.1007/BF00399108](https://doi.org/10.1007/BF00399108)
38. G. J. Abilay, M. R. Carroll, M. R. Palmer, J. Martí, R. S. J. Sparks, Basanite-phonolite lineages of the Teide-Pico volcanic complex, Tenerife, Canary Islands. *J. Petrol.* **39**, 905–936 (1998). doi: [10.1093/petroj/39.5.905](https://doi.org/10.1093/petroj/39.5.905)
39. P. M. Smith, P. D. Asimow, Adiabatic_1ph: A new public front-end to the MELTS, pMELTS, and pHMELTS models. *Geochim. Geophys. Geosyst.* **6**, Q02004 (2005). doi: [10.1029/2004GC000816](https://doi.org/10.1029/2004GC000816)
40. M. S. Ghiorso, R. O. Sack, Chemical mass transfer in magmatic processes. IV. A revised and internally consistent thermodynamic model for the interpolation and extrapolation of liquid-solid equilibria. *Contrib. Mineral. Petrol.* **119**, 197–212 (1995). doi: [10.1007/BF00307281](https://doi.org/10.1007/BF00307281)
41. H. S. Yoder, C. E. Tilley, Origin of basaltic magmas: An experimental study of natural and synthetic rock systems. *J. Petrol.* **3**, 342–532 (1962). doi: [10.1093/petrology/3.3.342](https://doi.org/10.1093/petrology/3.3.342)
42. D. C. Presnall *et al.*, Liquidus phase relations on the join diopside-forsterite-anorthite from 1-atm to 20 kbar: Their bearing on the generation and crystallization of basaltic magma. *Contrib. Mineral. Petrol.* **66**, 203–220 (1978). doi: [10.1007/BF00372159](https://doi.org/10.1007/BF00372159)
43. D. H. Eggler, Water-saturated and undersaturated melting relations in a Paricutin andesite and an estimate of water content in the natural magma. *Contrib. Mineral. Petrol.* **34**, 261–271 (1972). doi: [10.1007/BF00373757](https://doi.org/10.1007/BF00373757)
44. P. J. Wyllie, Magmas and volatile components. *Am. Mineral.* **64**, 469–500 (1979).
45. G. J. Abilay, G. G. J. Ernst, J. Martí, R. S. J. Sparks, The ~2ka subplinian eruption of Montana Blanca, Tenerife. *Bull. Volcanol.* **57**, 337–355 (1995).
46. C. Beier, K. M. Haase, T. H. Hansteen, Magma evolution of the Sete Cidades volcano, São Miguel, Azores. *J. Petrol.* **47**, 1375–1411 (2006). doi: [10.1093/petrology/egl014](https://doi.org/10.1093/petrology/egl014)

47. J. C. Dann, A. H. Holzheid, T. L. Grove, H. Y. McSweeney Jr., Phase equilibria of the Shergotty meteorite: Constraints on pre-eruptive water contents of martian magmas and fractional crystallization under hydrous conditions. *Meteorit. Planet. Sci.* **36**, 793–806 (2001). doi: [10.1111/j.1945-5100.2001.tb01917.x](https://doi.org/10.1111/j.1945-5100.2001.tb01917.x)
48. H. Nekvasil, J. Filiberto, F. M. McCubbin, D. H. Lindsley, Alkalic parental magmas for chassignites? *Meteorit. Planet. Sci.* **42**, 979–992 (2007). doi: [10.1111/j.1945-5100.2007.tb01145.x](https://doi.org/10.1111/j.1945-5100.2007.tb01145.x)
49. M. C. Johnson, M. J. Rutherford, P. C. Hess, Chassigny petrogenesis: Melt compositions, intensive parameters, and water contents of martian (?) magmas. *Geochim. Cosmochim. Acta* **55**, 349–366 (1991). doi: [10.1016/0016-7037\(91\)90423-3](https://doi.org/10.1016/0016-7037(91)90423-3)
50. F. M. McCubbin *et al.*, Hydrous magmatism on Mars: A source of water for the surface and subsurface during the Amazonian. *Earth Planet. Sci. Lett.* **292**, 132–138 (2010). doi: [10.1016/j.epsl.2010.01.028](https://doi.org/10.1016/j.epsl.2010.01.028)
51. J. Filiberto, A. H. Treiman, Martian magmas contained abundant chlorine, but little water. *Geology* **37**, 1087–1090 (2009). doi: [10.1130/G30488A.1](https://doi.org/10.1130/G30488A.1)
52. D. R. Baker, M. Alletti, Fluid saturation and volatile partitioning between melts and hydrous fluids in crustal magmatic systems: The contribution of experimental measurements and solubility models. *Earth Sci. Rev.* **114**, 298–324 (2012). doi: [10.1016/j.earscirev.2012.06.005](https://doi.org/10.1016/j.earscirev.2012.06.005)
53. M. R. Carroll, J. D. Webster, Solubilities of sulfur, noble gases, nitrogen, chlorine, and fluorine in magmas. *Rev. Mineral.* **30**, 231–279 (1994).
54. P. J. Wyllie, O. F. Tuttle, Experimental investigations of silicate systems containing two volatile components. Part III. The effects of SO₂, P₂O₅, HCl, and Li₂O, in addition to H₂O, on the melting temperatures of albite and granite. *Am. J. Sci.* **262**, 930–939 (1964). doi: [10.2475/ajls.262.7.930](https://doi.org/10.2475/ajls.262.7.930)
55. A. D. Edgar, in *Alkaline Igneous Rocks*, J. G. Fitton, B. G. J. Upton, Eds. (Geological Society of London, London, 1987), pp. 29–52.
56. D. H. Green, T. J. Falloon, W. R. Taylor, in *Magmatic Processes: Physicochemical Principles*, B. O. Mysen, Ed. (The Geochemical Society, University Park, PA, 1987), pp. 139–154.
57. R. Dasgupta, M. M. Hirschmann, N. D. Smith, Partial melting experiments of peridotite + CO₂ at 3 GPa and genesis of alkalic ocean island basalts. *J. Petrol.* **48**, 2093–2124 (2007). doi: [10.1093/petrology/egm053](https://doi.org/10.1093/petrology/egm053)
58. F. E. Lloyd, D. K. Bailey, Light element metasomatism of the continental mantle: The evidence and the consequences. *Phys. Chem. Earth* **9**, 389–416 (1975). doi: [10.1016/0079-1946\(75\)90030-0](https://doi.org/10.1016/0079-1946(75)90030-0)
59. M. Menzies, V. Rama Murthy, Mantle metasomatism as a precursor to the genesis of alkaline magmas—Isotopic evidence. *Am. J. Sci.* **280-A**, 622–638 (1980).
60. S. Pilet, M. B. Baker, E. M. Stolper, Metasomatized lithosphere and the origin of alkaline lavas. *Science* **320**, 916–919 (2008). doi: [10.1126/science.1156563](https://doi.org/10.1126/science.1156563); pmid: [18487189](https://pubmed.ncbi.nlm.nih.gov/18487189/)
61. S. Keshav, G. H. Gudfinnsson, G. Sen, Y. W. Fei, High-pressure melting experiments on garnet clinopyroxene and the alkalic to tholeiitic transition in ocean-island basalts. *Earth Planet. Sci. Lett.* **223**, 365–379 (2004). doi: [10.1016/j.epsl.2004.04.029](https://doi.org/10.1016/j.epsl.2004.04.029)
62. T. Kogiso, M. M. Hirschmann, D. J. Frost, High-pressure partial melting of garnet pyroxenite: Possible mafic lithologies in the source of ocean island basalts. *Earth Planet. Sci. Lett.* **216**, 603–617 (2003). doi: [10.1016/S0012-821X\(03\)00538-7](https://doi.org/10.1016/S0012-821X(03)00538-7)
63. M. E. Schmidt, T. J. McCoy, The evolution of a heterogeneous Martian mantle: Clues from K, P, Ti, Cr, and Ni variations in Gusev basalts and shergottite meteorites. *Earth Planet. Sci. Lett.* **296**, 67–77 (2010). doi: [10.1016/j.epsl.2010.04.046](https://doi.org/10.1016/j.epsl.2010.04.046)
64. H. Y. McSweeney Jr. *et al.*, Alkaline volcanic rocks from the Columbia Hills, Gusev crater, Mars. *J. Geophys. Res.* **111**, E09S91 (2006). doi: [10.1029/2006JE002698](https://doi.org/10.1029/2006JE002698)
65. G. Dreibus, H. Wänke, Volatiles on Earth and Mars: A comparison. *Icarus* **71**, 225–240 (1987). doi: [10.1016/0019-1035\(87\)90148-5](https://doi.org/10.1016/0019-1035(87)90148-5)
66. G. J. Taylor *et al.*, Bulk composition and early differentiation of Mars. *J. Geophys. Res.* **111**, E03S10 (2006).
67. K. Lodders, B. Fegley Jr., An oxygen isotope model for the composition of Mars. *Icarus* **126**, 373–394 (1997). doi: [10.1006/icar.1996.5653](https://doi.org/10.1006/icar.1996.5653)
68. S. M. McLennan, Large-ion lithophile element fractionation during the early differentiation of Mars and the composition of the Martian primitive mantle. *Meteorit. Planet. Sci.* **38**, 895–904 (2003). doi: [10.1111/j.1945-5100.2003.tb00286.x](https://doi.org/10.1111/j.1945-5100.2003.tb00286.x)
69. A. H. Treiman, Chemical compositions of martian basalts (shergottites): Some inferences on basalt formation, mantle metasomatism, and differentiation in Mars. *Meteorit. Planet. Sci.* **38**, 1849–1864 (2003). doi: [10.1111/j.1945-5100.2003.tb00019.x](https://doi.org/10.1111/j.1945-5100.2003.tb00019.x)
70. J. D. Winter, *An Introduction to Igneous and Metamorphic Petrology* (Prentice Hall, Upper Saddle River, NJ, 2010).
71. M. E. Schmidt *et al.*, APXS of first rocks encountered by Curiosity in Gale Crater: Geochemical diversity and volatile element (K and Zn) enrichment. *Lunar Planet. Sci. Conf. 44*, abstract 1278 (2013).
72. R. W. Le Maitre, Ed., *Igneous Rocks: A Classification and Glossary of Terms* (Cambridge Univ. Press, Cambridge, ed. 2, 2002).
73. H. C. Aoudjehane *et al.*, Tissint martian meteorite: A fresh look at the interior, surface, and atmosphere of Mars. *Science* **338**, 785–788 (2012). doi: [10.1126/science.1224514](https://doi.org/10.1126/science.1224514); pmid: [23065902](https://pubmed.ncbi.nlm.nih.gov/23065902/)
74. C. B. Agee *et al.*, Unique meteorite from early Amazonian Mars: Water-rich basaltic breccia Northwest Africa 7034. *Science* **339**, 780–785 (2013). doi: [10.1126/science.1228858](https://doi.org/10.1126/science.1228858); pmid: [23287721](https://pubmed.ncbi.nlm.nih.gov/23287721/)
75. D. W. Ming *et al.*, Geochemical properties of rocks and soils in Gusev crater, Mars: Results of the Alpha Particle X-ray Spectrometer from Cumberland Ridge to Home Plate. *J. Geophys. Res.* **113**, E12S39 (2008). doi: [10.1029/2008JE003195](https://doi.org/10.1029/2008JE003195)
76. R. Rieder *et al.*, Chemistry of rocks and soils at Meridiani Planum from the Alpha Particle X-ray Spectrometer. *Science* **306**, 1746–1749 (2004). doi: [10.1126/science.1104358](https://doi.org/10.1126/science.1104358); pmid: [15576611](https://pubmed.ncbi.nlm.nih.gov/15576611/)
77. S. W. Squyres *et al.*, Pyroclastic activity at Home Plate in Gusev crater, Mars. *Science* **316**, 738–742 (2007). doi: [10.1126/science.1139045](https://doi.org/10.1126/science.1139045); pmid: [17478719](https://pubmed.ncbi.nlm.nih.gov/17478719/)
78. R. H. Hewins *et al.*, Northwest Africa 7533, an impact breccia from Mars. *Lunar Planet. Sci. Conf. 44*, abstract 2385 (2013).
79. M. Humayun, B. Zanda, R. H. Hewins, C. Göpel, Composition of Northwest Africa 7533: Implications for the origin of martian soils and crust. *Lunar Planet. Sci. Conf. 44*, abstract 1429 (2013).
80. A. K. Matzen, M. B. Baker, J. R. Beckett, E. M. Stolper, Fe-Mg partitioning between olivine and high-magnesian melts and the nature of Hawaiian parental liquids. *J. Petrol.* **52**, 1243–1263 (2011). doi: [10.1093/petrology/egg089](https://doi.org/10.1093/petrology/egg089)
81. J. Filiberto, R. Dasgupta, Fe²⁺-Mg partitioning between olivine and basaltic melts: Applications to genesis of olivine-phyric shergottites and conditions of melting in the Martian interior. *Earth Planet. Sci. Lett.* **304**, 527–537 (2011). doi: [10.1016/j.epsl.2011.02.029](https://doi.org/10.1016/j.epsl.2011.02.029)

Acknowledgments: P. Antoshechkin provided insight concerning several aspects of MELTS and S. Lambart ran early MELTS calculations and provided initial compilations of terrestrial alkaline lavas. We would also like to thank two anonymous referees for constructive reviews of the manuscript and the MSL Project engineering and management teams for their efforts in making the mission such a success. This work was supported by grants from the NSF, NASA, the Canadian Space Agency, and the Centre National d'Études Spatiales. Compositional data for Jake_M are reported in Table 1.

Supplementary Materials
www.sciencemag.org/content/341/6153/1239463/suppl/DC1
 Materials and Methods
 Supplementary Text
 Figs. S1 to S24
 MSL Science Team Author List
 References (82–98)

22 April 2013; accepted 8 August 2013
[10.1126/science.1239463](https://doi.org/10.1126/science.1239463)

Soil Diversity and Hydration as Observed by ChemCam at Gale Crater, Mars

P.-Y. Meslin,^{1,2*} O. Gasnault,^{1,2} O. Forni,^{1,2} S. Schröder,^{1,2} A. Cousin,³ G. Berger,^{1,2} S. M. Clegg,³ J. Lasue,^{1,2} S. Maurice,^{1,2} V. Sautter,⁴ S. Le Mouéléc,⁵ R. C. Wiens,³ C. Fabre,⁶ W. Goetz,⁷ D. Bish,⁸ N. Mangold,⁵ B. Ehlmann,^{9,10} N. Lanza,³ A.-M. Harri,¹¹ R. Anderson,¹² E. Rampe,¹³ T. H. McConnochie,¹⁴ P. Pinet,^{1,2} D. Blaney,¹⁰ R. Léveillé,¹⁵ D. Archer,¹³ B. Barraclough,¹⁶ S. Bender,¹⁶ D. Blake,¹⁷ J. G. Blank,¹⁷ N. Bridges,¹⁸ B. C. Clark,¹⁹ L. DeFlores,¹⁰ D. Delapp,³ G. Dromart,²⁰ M. D. Dyar,²¹ M. Fisk,²² B. Gondet,²³ J. Grotzinger,⁹ K. Herkenhoff,¹² J. Johnson,¹⁸ J.-L. Lacour,²⁴ Y. Langevin,²³ L. Leshin,²⁵ E. Lewin,²⁶ M. B. Madsen,²⁷ N. Melikechi,²⁸ A. Mezzacappa,²⁸ M. A. Mischna,¹⁰ J. E. Moores,²⁹ H. Newsom,³⁰ A. Ollila,³⁰ R. Perez,³¹ N. Renno,³² J.-B. Sirven,²⁴ R. Tokar,¹⁶ M. de la Torre,⁹ L. d'Uston,^{1,2} D. Vaniman,¹⁶ A. Yingst,¹⁶ MSL Science Team†

The ChemCam instrument, which provides insight into martian soil chemistry at the submillimeter scale, identified two principal soil types along the Curiosity rover traverse: a fine-grained mafic type and a locally derived, coarse-grained felsic type. The mafic soil component is representative of widespread martian soils and is similar in composition to the martian dust. It possesses a ubiquitous hydrogen signature in ChemCam spectra, corresponding to the hydration of the amorphous phases found in the soil by the CheMin instrument. This hydration likely accounts for an important fraction of the global hydration of the surface seen by previous orbital measurements. ChemCam analyses did not reveal any significant exchange of water vapor between the regolith and the atmosphere. These observations provide constraints on the nature of the amorphous phases and their hydration.

The composition, mineralogy, and volatile inventory of the martian soil constitute an open record of the igneous history of the martian crust, its meteoritic bombardment, and the physical and chemical weathering processes that transformed primary igneous rocks into secondary products [e.g., (1–3)]. They reflect the aqueous history of Mars and the evolution of its climate. Physical weathering, transport, and sorting processes have redistributed crustal constituents in the soil, thus making its composition difficult to decipher by remote sensing observations. However, these processes also make the crust composition accessible through local in situ measurements.

From orbital observations (neutron, gamma-ray, and near-infrared spectroscopy), the martian surface is known to hold 2 to ~10 weight percent (wt %) of water-equivalent hydrogen at mid- and low latitudes (4–6). Thermodynamic models of ice stability [e.g., (7)] and equilibration models of various hydrous minerals [e.g., (8, 9)] have ruled out some simple explanations for the origin of this water reservoir. Although hydrated sulfates and clay minerals have been detected from orbit, they cover only a small fraction of the entire surface (10–12) and, to the extent of what can be observed by remote sensing, are limited to bedrock outcrops (13, 14), whereas the hydration of the topmost microns of the regolith probed by the Visible and Infrared Mineralogical Mapping Spectrometer (OMEGA) aboard the European Space Agency's Mars Express orbiter is

global and usually unrelated to bedrock exposures (6, 15). Therefore, the nature and origin of this global hydration remain largely unknown. Determining its nature is important for understanding the relationship between the regolith and the atmosphere as well as the aqueous history of Mars overall.

Before the Mars Science Laboratory mission, the bulk chemical composition of the soil was characterized in situ at five different locations on Mars by Viking 1 and 2 (16, 17), Mars Pathfinder (18), and the Mars Exploration Rovers (MERs) both at Meridiani Planum (19) and in Gusev Crater (20). Both bright dust and dark soil deposits on opposite sides of the planet were found to be very similar (21). The Gamma Ray Spectrometer (GRS) onboard the Mars Odyssey orbiter provided data about the elemental composition of the martian surface (4) and revealed large-scale heterogeneities, suggesting both local and regional sources for the surficial materials (22). Analyses of martian meteorites gave detailed information on the composition of the martian crust and interior (23). The composition of the martian surface was also estimated indirectly from its mineralogical characterization (24–26), but the cross section for very fine particles in these observations is often inordinately small. These investigations covered very different spatial scales, but no in situ information on martian soil chemistry was available at the subcentimeter scale (in areal extent), except for its volatile inventory in organic and volatile inorganic compounds by the

Viking and Phoenix landers (~100-mg samples were analyzed by the Viking Molecular Analysis Experiment) (27, 28). This higher resolution is crucial to unraveling the chemical and physical processes that formed the martian soil. Understanding the soil fine-scale chemistry, including its hydration, is an important objective of the ChemCam instrument onboard the Curiosity rover.

The laser-induced breakdown spectrometer (LIBS) on the ChemCam instrument (29, 30) provides insight on martian soil and dust chemical variability at the submillimeter scale. The small sampling area of the ChemCam laser (~350 to 550 μm depending on distance) allows it to isolate various soil components and identify mixing trends that bulk measurements might average together. The spectroscopic measurement of each individual soil, or “LIBS point” (31), is typically obtained from a series of 30 to 50 laser shots. Because each shot produces a LIBS spectrum of a deeper portion of the soil than the previous shot, it is possible to retrieve a profile of chemical composition to depths of a few millimeters in soils and a few tens of micrometers in rocks. The uncertainty budget of the LIBS measurements is small enough for such types of analyses to be performed. This yields analyses deeper than the probing depth of thermal, near-infrared, and x-ray spectrometers but shallower than GRS

¹Université de Toulouse, UPS-OMP, IRAP, 31028 Toulouse, France. ²CNRS, IRAP, 9 Av. Colonel Roche, BP 44346, F-31028 Toulouse cedex 4, France. ³Los Alamos National Laboratory, Los Alamos, NM 87545, USA. ⁴Muséum National d'Histoire Naturelle, Laboratoire de Minéralogie et Cosmochimie du Muséum, 75005 Paris, France. ⁵LPGN, CNRS, UMR6112, Université Nantes, 44322 Nantes, France. ⁶GeoRessources, CNRS, UMR7356, Université de Lorraine, 54506 Vandœuvre lès Nancy, France. ⁷Max Planck Institut für Sonnensystemforschung, 37191 Katlenburg-Lindau, Germany. ⁸Indiana University, Bloomington, IN 47405, USA. ⁹California Institute of Technology, Pasadena, CA 91125, USA. ¹⁰Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA. ¹¹Earth Observation Research Division, Finnish Meteorological Institute, 00101 Helsinki, Finland. ¹²U.S. Geological Survey, Astrogeology Science Center, Flagstaff, AZ 86001, USA. ¹³NASA Johnson Space Center, Houston, TX 77058, USA. ¹⁴University of Maryland, College Park, MD 20740, USA. ¹⁵Canadian Space Agency, Saint-Hubert, Quebec J3Y 8Y9, Canada. ¹⁶Planetary Science Institute, Tucson, AZ 85719, USA. ¹⁷NASA Ames Research Center, Moffett Field, CA 94035, USA. ¹⁸Applied Physics Laboratory, Johns Hopkins University, Laurel, MD 20723, USA. ¹⁹Space Science Institute, Boulder, CO 80301, USA. ²⁰ENS, 69007 Lyon, France. ²¹Mount Holyoke College, South Hadley, MA 01075, USA. ²²Oregon State University, Corvallis, OR 97331, USA. ²³Institut d'Astrophysique Spatiale, 91405 Orsay, France. ²⁴Commissariat à l'Energie Atomique et aux Energies Alternatives, Centre de Saclay, 91400 Gif-sur-Yvette, France. ²⁵Rensselaer Polytechnic Institute, Troy, NY 12180, USA. ²⁶ISTerre, 38041 Grenoble, France. ²⁷Niels Bohr Institute, University of Copenhagen, 2100 Copenhagen, Denmark. ²⁸Optical Science Center for Applied Research, Delaware State University, Dover, DE 19901, USA. ²⁹Center for Research in Earth and Space Science, York University, Toronto, Ontario M3J 1P3, Canada. ³⁰University of New Mexico, Albuquerque, NM 87131, USA. ³¹Centre National d'Etudes Spatiales, 31400 Toulouse, France. ³²Department of Atmospheric, Oceanic and Space Science, University of Michigan, Ann Arbor, MI 48109, USA.

*Corresponding author. E-mail: pmeslin@irap.omp.eu

†MSL Science Team authors and affiliations are listed in the supplementary materials.

Curiosity at Gale Crater

nuclear techniques, which can profile a few tens of centimeters. ChemCam is sensitive to the presence of hydrogen and can therefore investigate its spatial and temporal variability, providing constraints on the H₂O budget of the martian surface.

Results

With 139 LIBS points acquired on soil targets during the first 90 sols of the mission (~3600 spectra) (Fig. 1A), ChemCam has collected a data set that is well suited to statistical analysis. Here, a soil target is defined as a loose, unconsolidated material that can be distinguished from rocks, bedrock, or strongly cohesive sediments (32). Images taken with the Remote Microscopic Imager (RMI) show a diversity of soil targets analyzed by ChemCam that range from homogeneous soils made of fine-sand particles (Fig.

1C) to mixtures of fines and pebbles with typical grain sizes of <4 mm, classified as “fine gravels” in terms of the Wentworth-Krumbein scale (Fig. 1, B and D).

Soil Chemical Diversity

A cluster analysis of the spectra based on an independent components analysis (ICA) (33, 34) and chemical quantification obtained with a partial least-squares technique known as PLS2 (34–36) revealed that the soils observed during the first 90 sols at Gale crater follow a compositional trend between two major end members: a mafic component (cluster 1 or “mafic type”), and an alkali-, aluminum-, and silica-rich component (cluster 2 or “felsic type”). Cluster analysis reveals that two main groups of targets are indeed discriminated by their Si, Al, and Na components (Fig. 2 and Fig. 3A). Compositions obtained with PLS2 are

consistent with this analysis (Fig. 3B). A third cluster shares a relatively high Mg component with the mafic type but has a lower H and a higher Na component. Its composition is intermediate between the two former end members. The mean composition of cluster 1 is close to the APXS (Alpha Particle X-ray Spectrometer) composition of Portage (a soil target in a rover wheel scuff measured by both instruments) and to the composition of the dust measured by ChemCam on rock surfaces, although the latter was found to show less chemical variation (Table 1) (37). The felsic type is similar in composition to the high-Si minerals measured by ChemCam in neighboring rocks, such as Stark, a pitted, pumice-like rock, and Link, classified as a fine-pebble fluvial conglomerate (Fig. 3B) (38–40). Felsic-type soil targets are mostly found in the hummocky region in the vicinity of the landing site (Bradbury Rise)

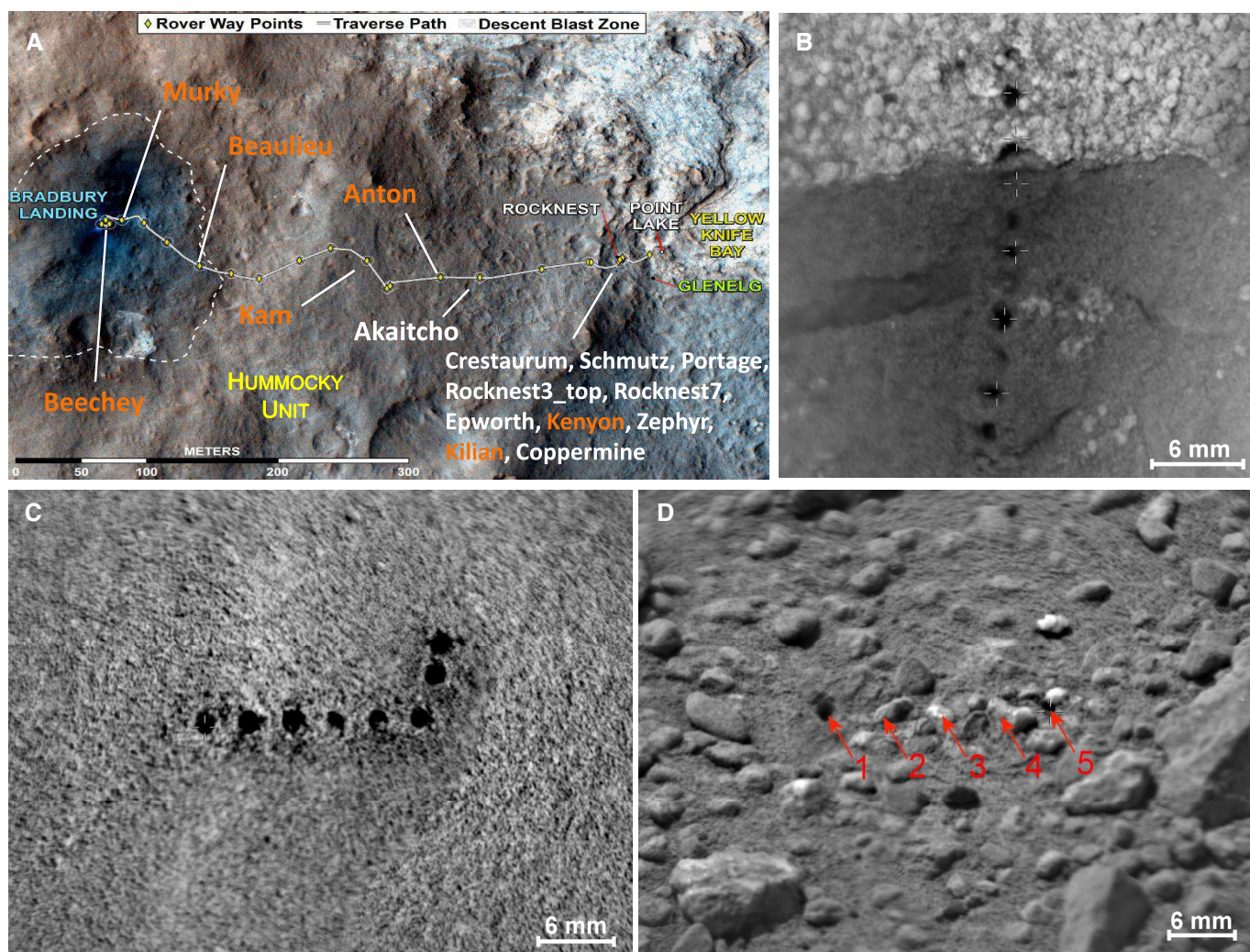


Fig. 1. ChemCam soil targets. (A) Rover traverse and location of ChemCam soil targets for the first 100 sols [image credit: NASA/JPL-Caltech/Univ. of Arizona]. Names appearing in orange correspond to locations where ChemCam points belonging to cluster 2 (felsic type) were found. Points belonging to cluster 1 (mafic type) were found in all locations. (B to D) Three examples of soil targets imaged by the RMI: (B) vertical transect across a trench dug into the Rocknest sand shadow, covered with ~1-mm grains (Epworth3, sol 84); 15 ChemCam points were acquired

from the bottom to the center of the image; the cross hairs only represent some of the LIBS spots; (C) homogeneous, fine-grained soil (Crestaurum, sol 83); (D) heterogeneous soil containing ~3-mm pebbles (Beaulieu, sol 33). The eight points of target Crestaurum and Beaulieu point #1 belong to cluster 1. Beaulieu points #2, #3, and #4 belong to cluster 2. Beaulieu point #5, at the intersection between a small pebble and the surrounding fines, belongs to cluster 3. Some points of Epworth, on top of the Rocknest sand shadow (B), also belong to cluster 3.

(Fig. 1A), which reveals a change in local source composition along the traverse. The composition of the average martian soil measured by other in situ missions (41) falls near the center of the Gale crater soil data cluster (Fig. 3B), closer to the mafic type, consistent with Mars Odyssey GRS data measured in these locations (22).

Relation Between Grain Size and Composition

Integrating physical properties such as grain size with chemical data is important to better understand the soil composition and its formation processes (3). Analyses of ChemCam RMI images and variations of chemical composition and intensity of the spectra with depth (fig. S1) (34) reveal a correlation between composition and grain size. The mafic-type soils comprise a mixture of grains that are both coarser and finer than the LIBS spot size of $\sim 400\text{ }\mu\text{m}$ (42), the coarser grains having on average higher SiO_2 and alkali abundances (Fig. 3B and fig. S3). Conversely, the felsic-type targets are almost exclusively coarse, millimeter-sized grains. This explains why cluster 2 is classified with some rock targets in the cluster analysis (Fig. 3A). The observed compositional trend, almost continuous, and its spread (Fig. 3B) could be produced by the mechanical mixing of different proportions of the two former components, from solid solutions or mixing between subcategories of each cluster, or from the presence of other chemical extremes falling on the same trend that could only be revealed by classification at the subpoint scale. Part of the spread

can also result from the inherent shot-to-shot dispersion of the spectra. Cluster 3 is an intermediate category of targets that is more difficult to define uniquely, probably as a result of the above processes and because the sorting of grains is less obvious. In some instances, clear mechanical mixing between the mafic and felsic types can be seen on RMI images of cluster 3 targets (Fig. 1D) or by looking at profiles of chemical composition with depth, which reveals the presence of buried coarse felsic grains (e.g., Epworth2 #3, located in the sand shadow armor shown in Fig. 1B).

Chemical Variability at Rocknest Site

The Curiosity rover remained for ~ 45 sols at a site called Rocknest in the vicinity of an aeolian bedform. The Rocknest soils, mostly sampled in the sand shadow, belong almost entirely to cluster 1. They differ distinctly from the iron-rich mafic rocks identified at Rocknest and are characterized by higher $\text{Mg}/(\text{Mn,Cr})$ (43), H, and Ca ICA components and lower Fe and Ti components than the latter (Fig. 3, A and C). The positive correlation between Mg and Cr suggests that they contain various proportions of picritic basaltic material. Although Mn and Cr are positively correlated with Mg in Rocknest soils, their concentration is lower than in the Rocknest rocks. These differences suggest an absence of a genetic relationship between soils and rocks in this area, except as noted below.

Spatial chemical diversity as a result of physical sorting by grain size (44) was readily appar-

ent in the sand shadow. Analysis points obtained on top of the bedform, armored with millimeter-sized grains (Fig. 1B), have on average higher Si, Na, K, and Al contents than the interior of the trench dug into the sand shadow, which is composed of fine-sand particles (fig. S3) (34). However, none of these points is classified with either felsic rocks/soils or Rocknest rocks, except Kenyon #8 (cluster 2) and Epworth2 #3 (classified in cluster 3 as a result of mixing between mafic type and felsic type), which suggests that they are not locally derived and have been subject to transport. The presence of the coarse grains Kenyon #8 and Epworth2 #3 provides evidence, however, that some local material has been incorporated into or on top of the sand shadow.

Low SiO_2 Abundances in the Fine-Grained Component

Some points within the mafic type (cluster 1), particularly its fine-grained fraction, have low SiO_2 (as low as $\sim 34\text{ wt } \%$) and a low sum of predicted oxides (Fig. 3B), averaging from $\sim 87\text{ wt } \%$ totals for the cluster 1 average composition to as low as $\sim 76\text{ wt } \%$ totals for the low SiO_2 value points. This suggests that the mafic-type soils contain a greater abundance of several elements (H, C, N, P, S, Cl, F) that are not easily detected by LIBS or quantifiable by PLS2. The low SiO_2 and total values may be attributed to the presence of an amorphous component that has been detected in the soil by CheMin and quantified by CheMin and APXS at levels of 27 to 45 wt % (45, 46). This component is Si-poor ($\text{SiO}_2 = 37.2\text{ wt } \%$) and S-, Cl-, and possibly P-rich (with values of $\text{SO}_3 = 11.0\text{ wt } \%$, $\text{Cl} = 1.4\text{ wt } \%$, and $\text{P}_2\text{O}_5 = 2.1\text{ wt } \%$) (46). Adding this composition from CheMin/APXS and 5 to 9 wt % of H_2O inferred from SAM (Sample Analysis at Mars) for the amorphous component (47) to the initial ChemCam totals yields a sum of $\sim 97.5\text{ wt } \%$ for the low-silica samples—a reasonable total given the accuracy of the PLS2 method (48) and the omission of other minor oxides in the calculation. It is also possible that lower SiO_2 values are associated with even greater abundances of S, Cl, and P than derived from the bulk values calculated or measured by CheMin and APXS. This analysis suggests that ChemCam has probed the soil amorphous component incorporated in the mafic type, which was made possible by the small area of the LIBS interaction.

It is also noteworthy that the fine-grained targets belonging to cluster 1 have higher CaO concentrations. PLS2 results reveal an anticorrelation between CaO and SiO_2 that mimics that between SiO_2 and the sum of missing oxides (Fig. 4), implying that a fraction of CaO is associated with an element that is not predicted by the PLS2 technique (49, 50). Some of this calcium could be associated with sulfur, which may be evidence for incorporation of Ca sulfates from neighboring areas. SAM and CheMin instruments have not found evidence for abundant crystalline Ca sulfate minerals in Rocknest soil ($<150\text{ }\mu\text{m}$ fraction),

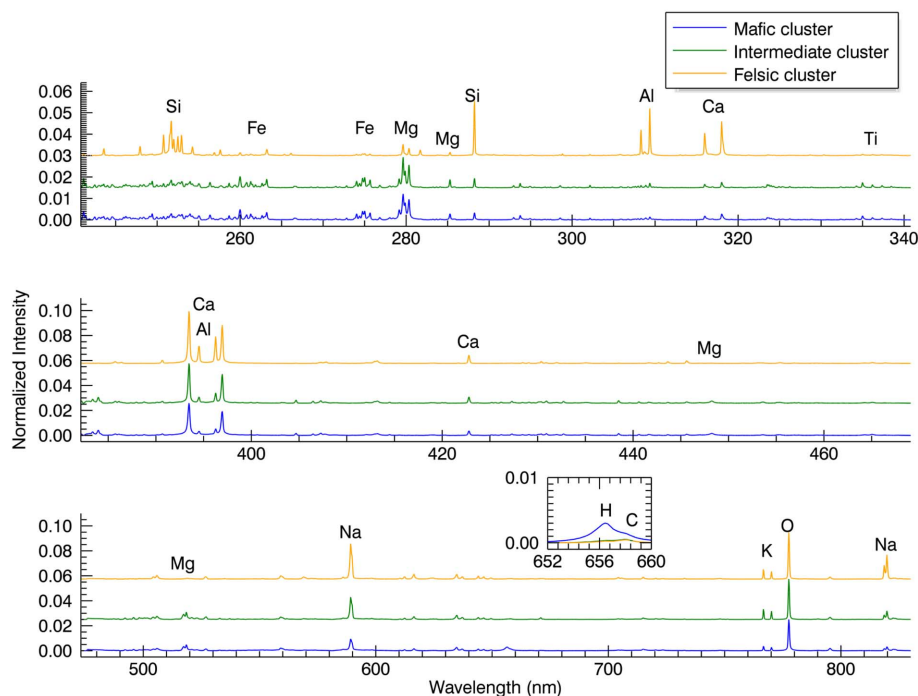


Fig. 2. Characteristic spectra of the three clusters. LIBS spectra of points Beaulieu #1 (mafic type), Beaulieu #2 (felsic type), and Beaulieu #5 (intermediate cluster), also shown in Fig. 1D. Each row corresponds to one of the three channels of ChemCam's Body Unit. An offset has been applied to separate the three spectra. The inset shows the hydrogen and carbon lines around 656 nm.

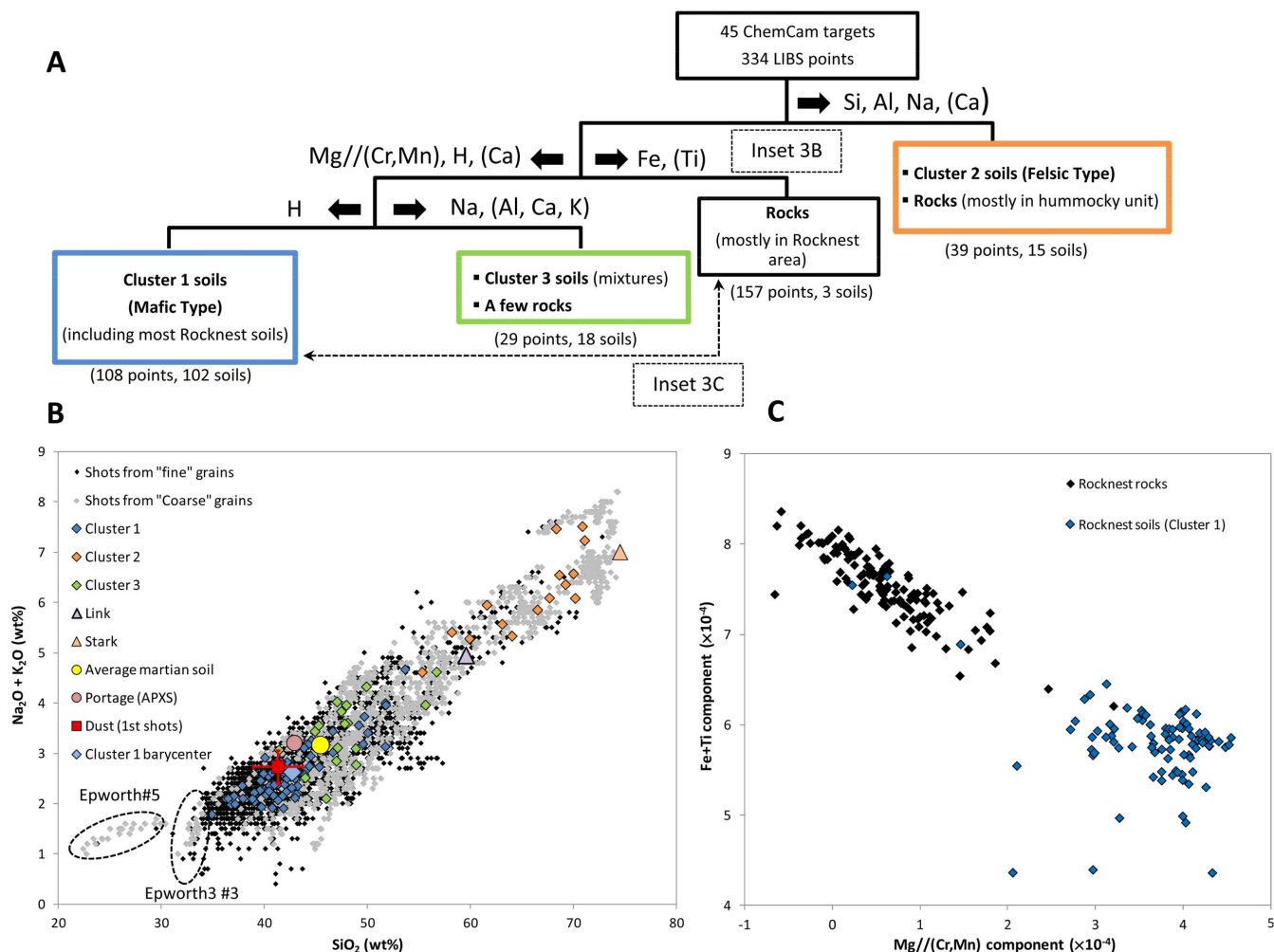


Fig. 3. Classification of ChemCam targets and chemical trends. (A) Schematic dendrogram of the clustering analysis of ChemCam targets (rocks and soils), based on ICA. The elements driving the division of the clusters are listed on each branch. The elements appearing in parentheses, although not sufficient to divide the clusters, show significantly larger signal than the mean of the whole population or subgroup. (B) Composition of ChemCam soil targets (the averages of each LIBS point are represented by colored diamonds), Link and Stark rocks, and dust, derived from the PLS2 technique. The dust composition is shown with $\pm 1\sigma$ standard deviation. All shot-by-shot data are

also shown, divided between shots that qualitatively appear to be from "coarse" grains (in gray) and from "fine" grains (in black). The average martian soil composition from (41) and the APXS composition of soil Portage (46) are shown for comparison. Differences in normalization are described in the supplementary material (34). (C) ICA classification of soils and rocks measured at Rocknest site showing a clear distinction between them along the (Fe+Ti) and Mg/(Cr,Mn) components (43). The point Epworth #5 (B), which represents a class in itself (characterized by its CaO component), is not represented in the dendrogram for the sake of clarity. Epworth3 #3 is another calcium-rich coarse grain.

although CheMin reports 1.4 wt % anhydrite, which is near its detection limits (45, 47). Ca perchlorates were identified tentatively by SAM at abundances below the detection limit of CheMin (45, 47). The largest CaO and lowest SiO₂ values were obtained for points Epworth #5 and Epworth3 #3 (Fig. 4), two coarse grains buried in the Rocknest sand shadow, whose size (>150 μ m) probably excluded them from being analyzed by SAM and CheMin.

Soil Hydration

An outstanding feature in ChemCam LIBS spectra of all cluster 1 soils is a ubiquitous hydrogen emission peak. ICA analysis reveals a hydration trend that closely follows the trend in composition and grain size (Fig. 5). The fine-grained mafic soil component and the dust (Fig. 6A) are

enriched significantly in H relative to the felsic-type component and coarse grains, whose H signal is similar to that of local rocks and ChemCam anhydrous calibration targets (Fig. 5 and Fig. 6A). This trend is consistent with the hypothesis that soils are a mechanical mixture of components characterized by different levels of hydration and that the average hydrogen abundance present corresponds to the proportion of the fine-grained component in the soil.

There are multiple potential carriers for this hydrogen: (i) adsorbed atmospheric H₂O, controlled by the soil specific surface area (SSA) and possibly forming thin grain-surface brines (51); (ii) hydrated crystalline minerals, including phyllosilicates and salts; and (iii) amorphous or poorly crystalline hydrated phases. CheMin found no

evidence for the presence of hydrated crystalline minerals in the soil, which suggests that the hydrogen detected by ChemCam and H₂O measured by SAM (47) is either adsorbed or corresponds to the hydration of the amorphous component detected by CheMin, or both. Adsorbed water would be preferentially associated with the amorphous phase if it is porous.

Three experiments were conducted with ChemCam to provide additional constraints on the nature of the observed hydrogen. The variability of hydrogen in the martian soil was monitored as a function of time and depth at different scales: (i) an examination of day/night H variations in the upper millimeter of undisturbed soil; (ii) observations of H variations with depth, over the first millimeter and over a few centi-

Table 1. Soils and dust composition and comparison to previous investigations. PLS2 mean compositions of clusters 1 and 2 (with standard deviations in parenthesis), dust (ChemCam first shots) (37), and comparison to the average martian soil (41) and to the APXS composition of Portage (46). PLS2 root-mean-square error of prediction (RMSEP) for each element is indicated in the rightmost

	Cluster 1 (mean)	Average martian soil*	Portage soil (ChemCam)	Portage soil (APXS)	Dust (ChemCam)	Dust (MER APXS)†	Cluster 2 (mean)	PLS2 RMSEP
SiO ₂	43.5 (3.8)	45.41	45.0 (4.4)	42.88 ± 0.47	42.0 (2.4)	44.84 ± 0.52	66.0 (5.0)	7.3
TiO ₂	0.6 (0.2)	0.90	0.6 (0.2)	1.19 ± 0.03	0.8 (0.2)	0.95 ± 0.08	0.1 (0.1)	0.7
Al ₂ O ₃	11.4 (1.1)	9.71	11.9 (1.4)	9.43 ± 0.14	10.9 (0.8)	9.32 ± 0.18	11.6 (1.6)	3.0
FeO _T	13.8 (1.1)	16.73	13.6 (1.5)	19.19 ± 0.12	13.7 (1.1)	16.96 ± 0.74	6.2 (3.7)	5.7
MgO	7.5 (1.5)	8.35	7.8 (1.8)	8.69 ± 0.14	7.3 (0.8)	7.89 ± 0.32	1.4 (1.1)	4.0
CaO	8.0 (1.4)	6.37	7.7 (1.8)	7.28 ± 0.07	7.8 (1.1)	6.34 ± 0.20	7.5 (2.7)	4.2
Na ₂ O	2.2 (0.4)	2.73	2.3 (0.5)	2.72 ± 0.10	2.0 (0.3)	2.56 ± 0.33	4.0 (0.4)	0.8
K ₂ O	0.6 (0.2)	0.44	0.6 (0.2)	0.49 ± 0.01	0.7 (0.2)	0.48 ± 0.07	2.1 (0.5)	0.9
Cr ₂ O ₃	—	0.36	—	0.49 ± 0.02	—	0.32 ± 0.04	—	—
MnO	—	0.33	—	0.41 ± 0.01	—	0.33 ± 0.02	—	—
P ₂ O ₅	—	0.83	—	0.94 ± 0.03	—	0.92 ± 0.09	—	—
SO ₃	—	6.16	—	5.45 ± 0.10	—	7.42 ± 0.13	—	—
Cl	—	0.68	—	0.69 ± 0.02	—	0.83 ± 0.05	—	—
Sum of oxides not quantified by PLS2		8.36		7.98		9.82		
Residual§	−3.0		−2.4		−4.2			
Total	87.6	99	89.5	99.85	85.2	99.2	98.9	11.4

*From (41). †From (54). ‡FeO = 10.42 ± 0.11 wt %, Fe₂O₃ = 7.28 ± 0.70 wt %. §Difference, expressed as [(total APXS) − (sum of oxides not quantified)] − (total PLS2), between adjacent columns. This residual is partly due to the difference of normalization, as APXS data are normalized on a water- and carbon-free basis (34).

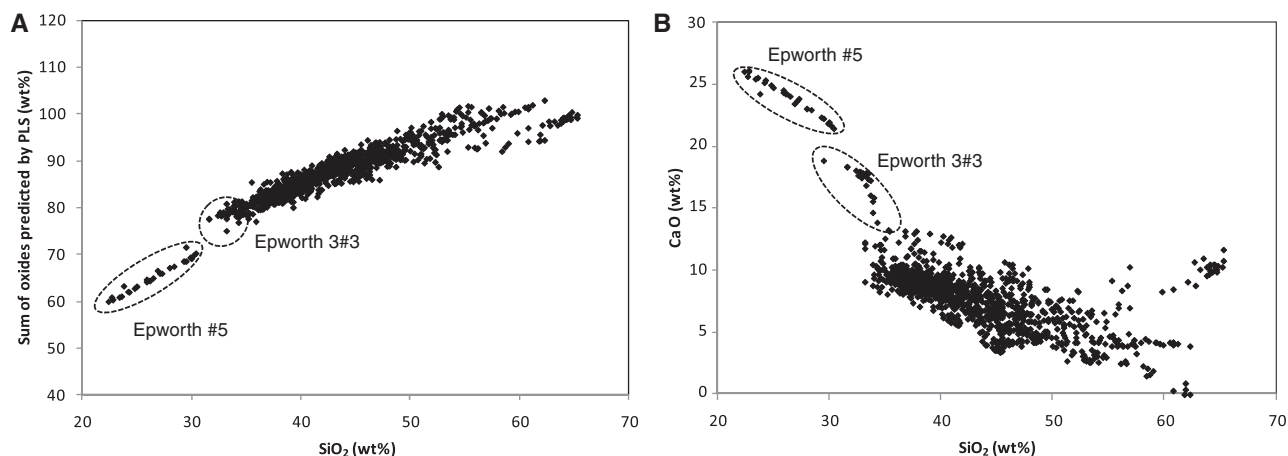


Fig. 4. Chemical correlations in the Rocknest soil. (A) Correlations between the sum of missing oxides and SiO₂, suggesting that the low SiO₂ values are associated with missing elements. (B) Anticorrelation between CaO and

SiO₂. Shot-by-shot PLS2 values of Rocknest soil are plotted together with shots from Epworth #5 and Epworth3 #3, two coarse grains found on top of and in the interior of the sand shadow, showing relatively large CaO abundances.

meters, when a scoop in the Rocknest sand shadow was excavated; and (iii) observations of H variations with time, over a few days, of a freshly exposed soil to look for signs of desiccation. The first experiment was performed on a fine-grained soil target, Crestaurum (Fig. 1C), at the surface of an aeolian bedform. Day and pre-dawn measurements of H showed very consistent values and no H enrichment in the first shots, which are most likely to be affected by diurnal exchange of H₂O or frost deposit (Fig. 6A). The second experiment was performed on a large set of points, both in undisturbed soil and in the interior of the ~3-cm-

deep trench in the Rocknest sand shadow; no statistically significant variations were seen within the first millimeter probed or between the different points (Fig. 6, B and D). This suggests that the H₂O content measured by SAM may be extrapolated to the surface of similar undisturbed soils. In the third experiment, similar points within the interior of the trench monitored over a 25-sol period, 11 sols after the soil was exposed, did not reveal statistically significant variations (Fig. 6C). These results, together with preliminary estimates of the sensitivity of ChemCam measurements (52), suggest that the diurnal exchange of H₂O with the atmosphere

leads to variations of H₂O abundance of less than ~1.1 wt % for the type of soils we analyzed under humidity conditions prevailing at Gale crater near solar longitude L_s ~ 200°. Either the equilibration of the interior of the sand shadow with the surface atmosphere occurred faster than 11 sols, or the gradient of water-equivalent hydrogen with depth was less than ~0.25 wt % over a few centimeters.

Discussion

Origin of the Soils

The abundance and distribution of light-toned pebbles with high Si, Na, and Al contents along

the rover traverse suggest that material with felsic composition is common near the landing site but limited to the pre-Rocknest hummocky unit. The surface of Bradbury Rise is characterized by the presence of fluvial sedimentary conglomerates, only one of which (Link) was observed by ChemCam (39). Its clasts have a range of SiO_2 , Al_2O_3 , and alkali compositions close to that of alkali feldspar (38–40), consistent with the composition of some felsic-type pebbles. This observation supports the interpretation that loose and cemented clasts in the hummocky region have a common origin. In this case, the felsic-type pebbles would have one of two origins: They could have resulted from in situ weathering of the sedimentary conglomerates, or they could have been transported and deposited from the same source region without being cemented. The abundance of such pebbles reinforces the view that the hummocky unit could contain abundant feldspar-rich crustal material that has not been probed by past instruments (38, 40) and possibly originates from the crater rim (39). The pebbles with largest SiO_2 concentrations may represent lithic fragments from silica-rich, pumice-like rocks similar to Stark (38, 40).

The mafic soil component was found not only in the Rocknest sand shadow and in aeolian bedforms, but throughout the rover traverse (Fig. 1A). It differs chemically from any of the rocks analyzed in the hummocky unit, supporting the idea that it is not locally derived at the scale of this geological unit. Its composition is similar to that of soils and dust measured elsewhere on Mars. Its presence in soils at Gale crater must reflect the efficiency of large-scale processes such as aeolian transport and impact gardening. It could reflect the widespread presence of regions with similar basaltic composition, possibly characterized by lower resistivity to physical and chemical weathering. On the other hand, investigations at other landing sites also found relatively little influence from local bedrock composition (21), rock compositions found at Gale crater differ from other sites (38), and Mars Odyssey GRS found large provinces characterized by different compositions (22, 53); these findings could indicate that the mafic soil component in fact represents an average of different compositions as a result of large-scale homogenization processes. It was suggested, for instance, that this ubiquitous material was made of two unrelated components: a component derived from relatively young olivine-rich basalts degraded under relatively anhydrous conditions and a component containing clay minerals, amorphous silica, and sulfur- and chlorine-rich nanophase ferric oxides resulting from the alteration of ancient rocks under hydrous conditions (2). This type of scenario could be consistent with ChemMin characterization of the Rocknest soil [although ChemMin did not detect the presence of phyllosilicates in that soil (45)] and with the evidence for the presence of a hydrated amorphous component.

The dust analyzed by ChemCam is basaltic, and its composition is similar to that measured by the MER APXS at the surface of bright soils (54)

(Table 1). Both its composition (including an SiO_2 abundance greater than that of the amorphous component) and its chemical homogeneity (i.e., small point-to-point variability, reflected in a standard deviation lower than that of cluster 1) suggest that its chemical alteration was limited, in agreement with previous observations of the presence of olivine minerals by the MERs (54–56). Fine by-products of the physical weathering of larger basaltic grains during their transport (57), or aeolian abrasion of rocks, would be consistent with these observations. On the other hand, the hydration measured by ChemCam, and the high S, P, and Cl content probably associated with nanophase iron oxides inferred from MER APXS analysis (54, 55), hint at the presence of weathering products, although adsorption could also play an important role given their small size. The homogeneity of the dust composition observed by ChemCam could therefore also reflect the very small scale of the dust particles with respect to the size of the LIBS spot.

The fact that none of the points analyzed on the Rocknest bedform armor is classified with Rocknest or Bradbury Rise rocks (except Kenyon #8) suggests that the millimeter-sized pebbles traveled some distance from another source region before ending their course at Rocknest. This is consistent with their subangular to subrounded shape, which is indicative of the mechanical erosion they have undergone (fig. S2).

Hydration of the Amorphous Phase and Specific Surface Area of the Soil

Although the nature of the amorphous component detected by ChemMin in the $<150\text{-}\mu\text{m}$ fraction of the Rocknest soil remains unclear, its x-ray diffraction pattern resembles that of basaltic glass with some allophane, a short-range ordered hydrous aluminosilicate (45). Chemical data, however, suggest that the latter is likely a surrogate for Fe^{3+} -bearing amorphous phases, such as Fe-allophane, hisingerite (58), or nanophase iron oxides (45, 46). Interpretations of orbital data also suggest the presence of allophane-like silicate phases and ferrihydrite at the surface of Mars (26, 59, 60), although Al-rich allophane is not consistent with APXS chemical data (46) and requires conditions of moderate pH (≥ 5) to form (61).

The large fraction of poorly ordered ferric materials and their association with hydrogen and other volatile species suggest a similarity to terrestrial andisols. These soils develop in volcanic ejecta (such as volcanic ash, pumice, or cinders) and/or in volcanoclastic materials usually rich in volcanic glass; they are characterized by a colloidal fraction dominated by short-range ordered minerals or noncrystalline phases, especially allophane, imogolite, and noncrystalline oxyhydroxides (e.g., ferrihydrite), or Al/Fe-humus complexes often together with opaline silica (61, 62). The strong sorption capacity of andisols

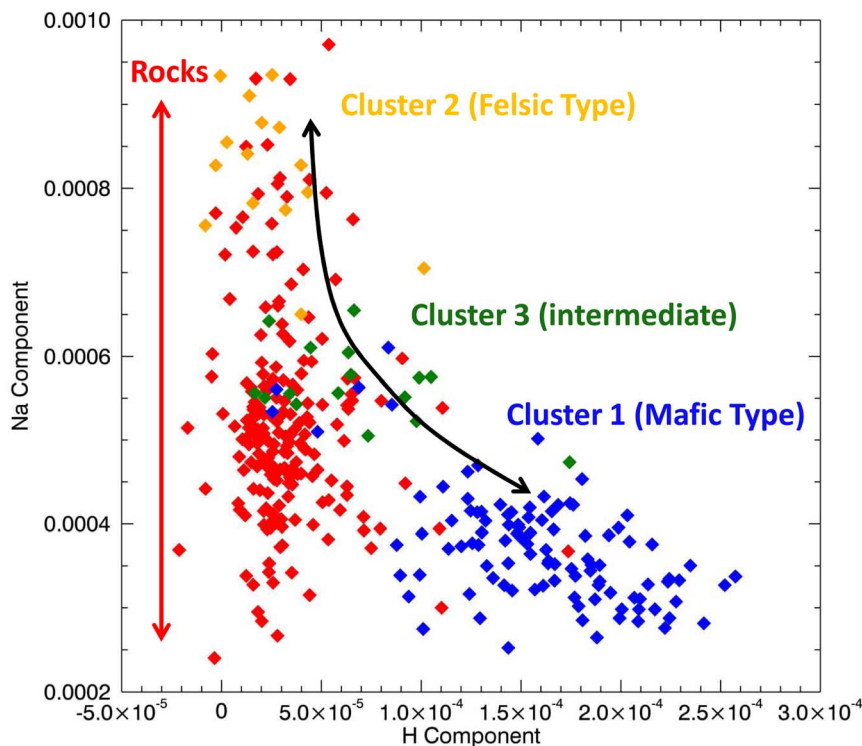


Fig. 5. ICA classification of soils and rocks along Na and H components. A hydration trend from cluster 2 to cluster 1 soils and going through cluster 3 is observed, away from the rocks (the x and y axis represent the covariance between each of the spectra and the independent components) (34). It suggests mechanical mixing between fine hydrated particles and drier coarse grains.

due to the presence of these amorphous phases could be relevant to Mars. Sorption of phosphate in andisols, for instance, has been shown to inhibit the crystallization of ferrihydrite to more crystalline goethite and hematite (61). High sorption capacities could also make the regolith an important contributor to the atmospheric H₂O cycle (63, 64). In andisols, allophane and iron (oxy)hydroxides contain substantial amounts of adsorbed H₂O because of their large specific surface area (SSA) (65) and the presence of surface hydroxyl groups that act as strong adsorption sites through hydrogen bonding (66). Similarly, the abundance of these hydroxylated phases may dominate the SSA of the martian soil and thus the level of atmospheric H₂O stored in the regolith. Laboratory experiments per-

formed on ferrihydrite and on phyllosilicate-poor, allophane-rich palagonitic dust from the flanks of Mauna Kea volcano, Hawaii (67, 68), show indeed that these materials can hold relatively large amounts of adsorbed H₂O (a few wt %) under martian conditions, even at low relative humidities and with relatively fast exchange kinetics (69).

Some differences with the martian soil are therefore unclear. Indeed, the average level of hydration of the amorphous phases (5 to 9 wt %), measured by SAM (47) to a temperature of 835°C, contrasts with the relatively small amount of H₂O measured at temperatures less than 150° to 200°C, a typical range of temperatures where H₂O adsorbed in allophane-rich material is released (70). This also contrasts with the lim-

ited time variability of H₂O abundance measured by ChemCam in the near-subsurface and inferred from orbital observations of the atmospheric water column (71).

Determining the level of regolith-atmosphere exchange of H₂O, and thus its SSA, is also important to understand why the D/H ratio of soils, measured by SAM, is close to atmospheric values (26). Estimates of the SSA of the martian soil can be deduced from ChemCam day/night experiment results (see Materials and Methods). We find an upper limit for the SSA of the fine-grained component of 30 to 45 m² g⁻¹ (Fig. 7) (34), which does not contradict the only SSA estimate available to date of 17 m² g⁻¹ derived by the Viking Gas Exchange Experiment (72). The relatively low SSA suspected for the

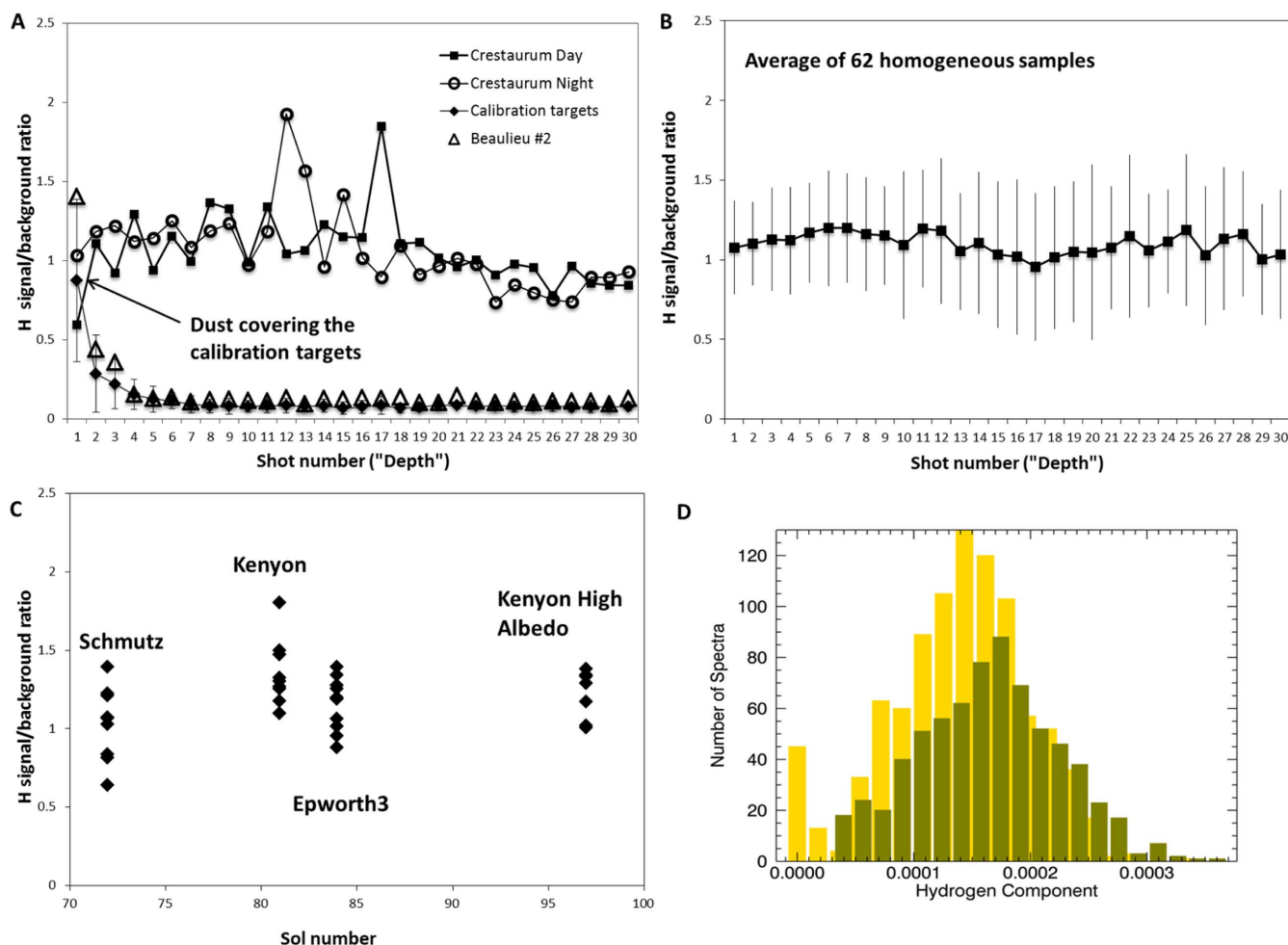


Fig. 6. Temporal and spatial variability of the hydrogen signal. (A) Depth profile of the H line intensity (signal to background, S/B) for the Crestaurum target analyzed on sol 74/75 [sol 74, 11:30 a.m. local mean solar time (LMST), and pre-dawn of sol 75, 4:40 a.m. LMST]. The ratio between the mean night and day S/B is 1.003, indicating no sign of water enrichment at night. The average S/B of seven onboard calibration targets, used as a blank, is also displayed. The first five shots reveal the presence of hydrated dust on their surface. The large error bars for these shots are probably due to differences in dust coverage. The H S/B profile of Beaulieu point #2 (Fig. 1D), which belongs to the felsic type, is also shown for comparison. It does not significantly differ

from the blank. (B) Hydrogen S/B ratio over the first 30 shots and averaged over 62 homogeneous, fine-grained samples. Error bars are $\pm 1\sigma$ (standard deviation of the N averaged samples). (C) Variations of the hydrogen S/B ratio in the interior of the trench as a function of the sol number (the trench was dug on sol 61). Each black point represents the average S/B of one of the LIBS points acquired on the target. The standard deviation around the mean of each target is ~ 0.2 . (D) Histograms of the two populations of spectra acquired in the interior of the trench (green) and in undisturbed surfaces (yellow), and characterized by the same Na component. The x axis represents the H component of the ICA analysis.

noncrystalline fraction of the soil, deduced from the low content of exchangeable H₂O and compared with the much higher values measured for terrestrial allophane and ferrihydrite, suggests that such strongly hydroxylated silicates may in fact not be a very adequate analog for the martian soil, or that their porous structure has been modified.

Conclusion

ChemCam and APXS instruments show that the fine-grained soil component measured at Gale crater is likely representative of widespread martian soils, owing to chemical similarity with the soils of other landing sites. It has been sampled not only in undisturbed soils along the rover traverse but also at some depth in Rocknest sand shadow. This component and the dust are found to possess a ubiquitous H emission line in ChemCam spectra. The corresponding hydration, quantified by SAM at Rocknest at a bulk value of ~2.25 wt % (47), relatively consistent with H₂O abundances of 1 to 3 wt % measured by Viking 1 and 2 (73) and shown by CheMin to be likely carried by amorphous phases (45, 46), could therefore account for an important fraction of the global budget of the water-equivalent hydrogen measured from orbit at mid- and equatorial latitudes by Mars Odyssey GRS and Mars Express OMEGA, in particular the lower limit of ~2 wt % (4–6, 15). Because the hydration of the amorphous component is ~5 to 9 wt % (47), the spatial variations seen from orbit may be part-

ly explained by the presence of different proportions of this hydrated amorphous component in the soil.

Materials and Methods

Constraints on the Specific Surface Area of the Martian Soil at Gale Crater

The SSA of a soil is the primary parameter that controls the amount of H₂O adsorbed onto it. Determining the SSA and the adsorption capacity of the soil is important to better understand the gaseous exchange between the regolith and the atmosphere, and it is also useful to constrain the nature of the hydrated amorphous phases measured by CheMin. As an illustration, in allophane, characteristic of andisols, H₂O molecules are bound strongly to hydroxyl functional groups such as Si-OH and Al-OH-Al, if those are accessible to H₂O molecules (66). Hydroxyl functional groups thus increase the ability of this material to adsorb water even at low relative humidities (RHs). Typically, allophane at RH = 30% can adsorb twice as much H₂O as its structural hydroxyl content (70). This is also the case for the popular allophane-rich martian analog JSC Mars-1 (74).

For a given SSA, the amount of water vapor adsorbed depends on the RH, as expressed by adsorption isotherms. On sol 74/75, the dates of the day/night experiment described above, the RH observations by the REMS-H device (Rover Environment Monitoring Station–Humidity Sensor) (75) in the early morning before sunrise gave

a preliminary value of ~20% at an altitude of 1.5 m above the surface. This corresponds to an RH value of 25 to 35% at the ground level because early-morning ground temperature is 1 to 3 K lower (76) than the atmospheric temperature at the MSL boom level. Noontime RH at sol 74 was approximately 0 to 0.05% because of the noontime high atmospheric temperature.

Estimates of the SSA can be obtained using adsorption isotherms obtained on martian analogs. Adsorption isotherms were measured for several geological samples under martian conditions ($T = 243$ K, $RH = 0$ to 70%), together with kinetic parameters (67, 69). Figure 7 shows that to first order, the amount of H₂O adsorbed is approximately linearly related to the SSA. The relative insensitivity to mineralogy for geological materials was also shown by (77). To hold <1 wt % of adsorbed H₂O at $RH = 0.001$, the SSA should be <13 m² g⁻¹. Similarly, to hold <1 wt % of adsorbed H₂O at $RH = 0.3$, the SSA should be <24 m² g⁻¹. Between $RH = 0.001$ and $RH = 0.3$, the differential amount of adsorbed water is ~0.033 wt %/(m² g⁻¹), corresponding to the different slopes in Fig. 7. Because ChemCam did not observe diurnal variations greater than ~1.1 wt %, the SSA should be lower than ~30 m² g⁻¹. Extrapolation of the adsorption isotherms from 243 K to temperatures measured at Gale crater at night is not believed to significantly affect this upper limit, either making it a safe upper limit or possibly increasing it to ~45 m² g⁻¹, depending on the isosteric heat of adsorption considered (34). Using the desorption branch of the isotherms published by (67) gave very similar results.

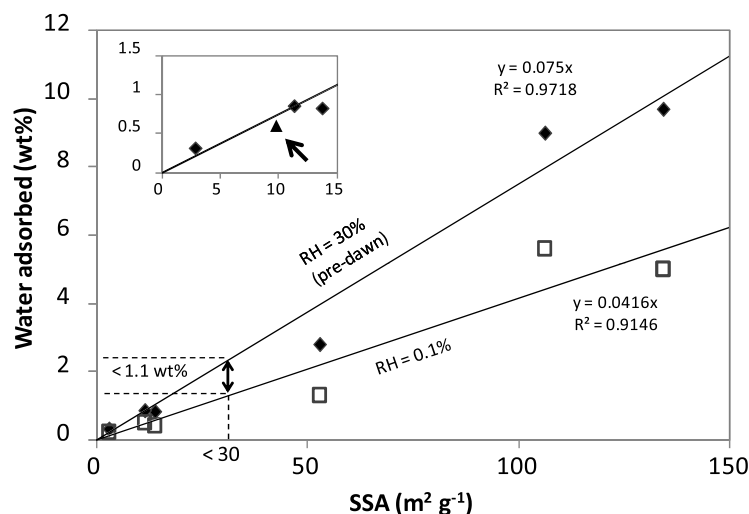


Fig. 7. Amount of adsorbed water as a function of SSA and relative humidity. The two curves represent linear fits through the experimental data (adsorption isotherms) measured by (67) on six geological samples at 243 K, for two values of RH (0.1% and 30%) corresponding to the conditions prevailing approximately at Gale during the day/night Crestaaurum experiment. Note that although there is some dispersion around the fits, the gaps between the experimental values and the fits (which matter here) are very similar. The double-sided arrow represents the upper limit of the difference of H₂O abundance estimated by ChemCam between the two local times, which translates into an upper limit for the SSA. By increasing order of SSA, the samples are as follows: dunite (2.83 m² g⁻¹), volcanic tuff + Mg sulfates (11.3 m² g⁻¹), volcanic tuff (13.7 m² g⁻¹), smectite SWy-2 (52.7 m² g⁻¹), JSC Mars-1 (106 m² g⁻¹), and ferrihydrite (134 m² g⁻¹). The experimental data of (78) (arrow in the inset) obtained at 253 K with ground Vacaville basalt (2.83 m² g⁻¹) fall well on the fit (no data available at low RH). See supplementary material for more details.

Spatial and Temporal Variability of the Hydrogen Signal

In order to investigate the variability of the H signal at different time and depth scales, three types of ChemCam experiments were conducted. First, we checked whether there was any gradient with depth within the first 30 shots, corresponding roughly to the first few millimeters of the subsurface. For that, a sample of 62 individual LIBS points has been analyzed. The choice of these specific points was dictated by the need to compare relatively similar, fine-grained soils. All were selected from cluster 1. The profile of each point was checked for the presence of any obvious “coarse” grains, and when it occurred, the corresponding shots were removed from the depth profile. Figure 6B shows the depth profile of the average signal-to-background (S/B) ratio of these 62 points. Within the uncertainty of the measurement (Fig. 6B), no trend with depth is observed.

To check whether the interior of the trench had a larger hydrogen signature than the exposed surface, given the lower maximal temperatures reached during the day, two sets of individual spectra were selected and compared: ~1000 spectra acquired on undisturbed soils and ~700 spectra acquired in the interior of the trench. Given the size of the data set, we preferred to perform an ICA analysis of the H component. However, it

was shown that all points plot on a mixing line between (high Na, low H) and (low Na, high H) (Fig. 5). Thus, to be comparable, the samples should have a similar Na component. The spectra corresponding to the fine-grained soil samples were selected in a range of low Na component values [the Mg/(Cr,Mn) component was also tested and gave exactly the same result]. The histograms showing the distribution of the H component for these two populations are presented in Fig. 6D. A Kolmogorov-Smirnov statistical test showed that there was no significant difference between the two populations. Therefore, we conclude that the interior of the trench does not show evidence of an H₂O enrichment compared to the undisturbed surface, within the sensitivity of the measurement (34). This has an implication with regard to the relevance of the extrapolation of the SAM results to a more global scale. It means that the hydrogen content measured by SAM is comparable to the hydrogen measured over an exposed surface, typically seen by orbital measurements. We do not see evidence for a two-layer model at this scale (a few centimeters). Nonetheless, this analysis does not rule out the possibility that the interior of the trench was more hydrated when it was excavated, and it lost its additional H₂O in the 11 sols between the scooping and the first measurement by ChemCam on sol 72.

Finally, we have monitored the evolution of the H signal of a freshly exposed soil (the trench in Rocknest ripple) over a 25-sol time span, 11 sols after it was scooped. The intent was to detect a possible desiccation of the exposed material. The average hydrogen S/B ratio of each LIBS point obtained for a series of four targets is plotted in Fig. 6C as a function of time, the *x* axis representing the sol at which the targets were measured: Schmutz (9 LIBS points, on sol 72), Kenyon (10 LIBS points, on sol 81), Epworth3 (12 first LIBS points, on sol 84), and Kenyon_high_albedo (10 LIBS points, on sol 97). Again, for each point, spectra attributed to “coarse” grains were removed. No statistically significant variation of the H signal was observed with time.

References and Notes

- M. H. Carr, *The Surface of Mars* (Cambridge Univ. Press, Cambridge, 2007).
- H. Y. McSweeney Jr., I. O. McGlynn, A. D. Rogers, Determining the modal mineralogy of Martian soils. *J. Geophys. Res.* **115**, E00F12 (2010). doi: [10.1029/2010JG003582](#)
- I. O. McGlynn, C. M. Fedo, H. Y. McSweeney Jr., Soil mineralogy at the Mars Exploration Rover landing sites: An assessment of the competing roles of physical sorting and chemical weathering. *J. Geophys. Res.* **117**, E01006 (2012). doi: [10.1029/2011JG003861](#)
- W. V. Boynton *et al.*, Concentration of H, Si, Cl, K, Fe, and Th in the low- and mid-latitude regions of Mars. *J. Geophys. Res.* **112**, E12599 (2007). doi: [10.1029/2007JG002887](#)
- S. Maurice *et al.*, Mars Odyssey neutron data: 1. Data processing and models of water-equivalent-hydrogen distribution. *J. Geophys. Res.* **116**, E11008 (2011). doi: [10.1029/2011JG003810](#)
- R. E. Milliken *et al.*, Hydration state of the Martian surface as seen by Mars Express OMEGA: 2. H₂O content of the surface. *J. Geophys. Res.* **112**, E08S07 (2007). doi: [10.1029/2006JG002853](#)
- O. Aharonson, N. Schorghofer, Subsurface ice on Mars with rough topography. *J. Geophys. Res.* **111**, E11007 (2006). doi: [10.1029/2005JG002636](#)
- T. Tokano, D. Bish, Hydration state and abundance of zeolites on Mars and the water cycle. *J. Geophys. Res.* **110**, 2156–2202 (2005). doi: [10.1029/2005JG002410](#)
- C. I. Fialips *et al.*, Hydration state of zeolites, clays, and hydrated salts under present-day Martian surface conditions: Can hydrous minerals account for Mars Odyssey observations of near-equatorial water-equivalent hydrogen? *Icarus* **178**, 74–83 (2005). doi: [10.1016/j.icarus.2005.04.020](#)
- F. Poulet *et al.*, Phyllosilicates on Mars and implications for early martian climate. *Nature* **438**, 623–627 (2005). doi: [10.1038/nature04274](#); pmid: [16319882](#)
- J. F. Mustard *et al.*, Hydrated silicate minerals on Mars observed by the Mars Reconnaissance Orbiter CRISM instrument. *Nature* **454**, 305–309 (2008). doi: [10.1038/nature07097](#); pmid: [18633411](#)
- J. Carter, F. Poulet, J.-P. Bibring, N. Mangold, S. Murchie, Hydrous minerals on Mars as seen by the CRISM and OMEGA imaging spectrometers: Updated global view. *J. Geophys. Res.* **118**, 831–858 (2013). doi: [10.1029/2012JG004145](#)
- J. Carter, F. Poulet, J. P. Bibring, S. Murchie, Detection of hydrated silicates in crustal outcrops in the northern plains of Mars. *Science* **328**, 1682–1686 (2010). doi: [10.1126/science.1189013](#); pmid: [20576889](#)
- B. L. Ehlmann *et al.*, Subsurface water and clay mineral formation during the early history of Mars. *Nature* **479**, 53–60 (2011). doi: [10.1038/nature10582](#); pmid: [22051674](#)
- D. Jouglet *et al.*, Hydration state of the Martian surface as seen by Mars Express OMEGA: 1. Analysis of the 3 μ m hydration feature. *J. Geophys. Res.* **112**, E08S06 (2007). doi: [10.1029/2006JG002846](#)
- B. C. Clark *et al.*, The Viking X ray fluorescence experiment: Analytical methods and early results. *J. Geophys. Res.* **82**, 4577–4594 (1977). doi: [10.1029/J5082i028p04577](#)
- B. C. Clark *et al.*, Chemical composition of martian fines. *J. Geophys. Res.* **87**, 10059–10067 (1982). doi: [10.1029/JB087iB12p10059](#)
- H. Wänke, J. Brückner, G. Dreibus, R. Rieder, I. Ryabchikov, Chemical composition of rocks and soils at the Pathfinder site. *Space Sci. Rev.* **96**, 317–330 (2001). doi: [10.1023/A:1011961725645](#)
- R. Rieder *et al.*, Chemistry of rocks and soils at Meridiani Planum from the Alpha Particle X-ray Spectrometer. *Science* **306**, 1746–1749 (2004). doi: [10.1126/science.1104358](#); pmid: [15576611](#)
- R. Gellert *et al.*, Chemistry of rocks and soils in Gusev Crater from the alpha particle x-ray spectrometer. *Science* **305**, 829–832 (2004). doi: [10.1126/science.1099913](#); pmid: [15297665](#)
- A. S. Yen *et al.*, An integrated view of the chemistry and mineralogy of martian soils. *Nature* **436**, 49–54 (2005). doi: [10.1038/nature03637](#); pmid: [16001059](#)
- O. Gasnault *et al.*, Quantitative geochemical mapping of Martian elemental provinces. *Icarus* **207**, 226–247 (2010). doi: [10.1016/j.icarus.2009.11.010](#)
- H. Y. McSweeney Jr., What we have learned about Mars from SNC meteorites. *Meteoritics* **29**, 757–779 (1994). doi: [10.1111/j.1945-5100.1994.tb01092.x](#)
- P. R. Christensen, J. L. Bandfield, M. D. Smith, V. E. Hamilton, R. N. Clark, Identification of a basaltic component on the Martian surface from Thermal Emission Spectrometer data. *J. Geophys. Res.* **105**, 9609–9621 (2000). doi: [10.1029/1999JG001127](#)
- A. D. Rogers, O. Aharonson, Mineralogical composition of sands in Meridiani Planum determined from Mars Exploration Rover data and comparison to orbital measurements. *J. Geophys. Res.* **113**, E06S14 (2008). doi: [10.1029/2007JG002995](#)
- F. Poulet *et al.*, Abundance of minerals in the phyllosilicate-rich units on Mars. *Astron. Astrophys.* **487**, L41–L44 (2008). doi: [10.1051/0004-6361/200810150](#)
- K. Biemann *et al.*, Search for organic and volatile inorganic compounds in two surface samples from the chryse planitia region of Mars. *Science* **194**, 72–76 (1976). doi: [10.1126/science.194.4260.72](#); pmid: [17793082](#)
- P. H. Smith *et al.*, H₂O at the Phoenix landing site. *Science* **325**, 58–61 (2009). pmid: [19574383](#)
- S. Maurice *et al.*, The ChemCam Instrument Suite on the Mars Science Laboratory (MSL) Rover: Science objectives and mast unit description. *Space Sci. Rev.* **170**, 95–166 (2012). doi: [10.1007/s11214-012-9912-2](#)
- R. Wiens *et al.*, The ChemCam instrument suite on the Mars Science Laboratory (MSL) rover: Body unit and combined system tests. *Space Sci. Rev.* **170**, 167–227 (2012). doi: [10.1007/s11214-012-9902-4](#)
- Hereafter, a “shot” corresponds to a single spectrum, and a “LIBS point” is a series of spectra acquired at the same laser ablation crater (i.e., sampling location).
- No implication of the presence or absence of organic materials or living matter is intended, nor is the genesis of the deposit.
- O. Forni *et al.*, Independent component analysis classification of laser induced breakdown spectroscopy spectra. *Spectrochim. Acta B* **86**, 31–41 (2013). doi: [10.1016/j.sab.2013.05.003](#)
- See supplementary materials on Science Online.
- S. Clegg, E. Sklute, M. D. Dyar, J. E. Barefield, R. C. Wiens, Multivariate analysis of remote laser-induced breakdown spectroscopy spectra using partial least squares, principal component analysis, and related techniques. *Spectrochim. Acta B* **64**, 79–88 (2009). doi: [10.1016/j.sab.2008.10.045](#)
- R. C. Wiens *et al.*, Pre-flight calibration and initial data processing for the ChemCam laser-induced breakdown spectroscopy instrument on the Mars Science Laboratory rover. *Spectrochim. Acta B* **82**, 1–27 (2013). doi: [10.1016/j.sab.2013.02.003](#)
- The composition of the dust was derived from the average of all the first spectra acquired on rock targets, which are very often different from the spectra of the underlying material and are found to be very homogeneous. They also share with soils a strong H signature. A small contamination of the underlying material in the first ablation cannot be totally ruled out.
- R. C. Wiens *et al.*, Compositions determined by ChemCam along Curiosity’s traverse from Bradbury station to Glenelg in Gale crater, Mars. *Lunar Planet. Sci. Conf.* **44**, abstract 1363 (2013).
- R. M. E. Williams *et al.*, Martian fluvial conglomerates at Gale crater. *Science* **340**, 1068–1072 (2013). doi: [10.1126/science.1237317](#); pmid: [23723230](#)
- V. Sautter *et al.*, Igneous composition variations determined by ChemCam along Curiosity’s traverse from Bradbury to Rocknest area at Gale crater, Mars. *Geophys. Res. Abstr.* **15**, EGU2013–12753 (2013).
- S. R. Taylor, S. M. McLennan, *Planetary Crusts: Their Composition, Origin, and Evolution* (Cambridge Univ. Press, Cambridge, 2009).
- These grains will be qualified qualitatively as “coarse” in the following analysis, in contrast to samples whose spectral features are typical of material that is fine-grained at the LIBS scale (34). The beam size is ~400 μ m at 2.8 m, average distance to the soil targets analyzed with ChemCam.
- The notation “//” means that the ICA component relates to the Mg abundance, not to the abundance of Cr and Mn, and indicates that the latter elements are positively correlated with Mg in Rocknest soils.
- G. Kocurek *et al.*, Rocknest sand shadow at the Curiosity field site: Morphology, origin, and stabilization. *Lunar Planet. Sci. Conf.* **44**, abstract 1375 (2013).
- D. L. Bish *et al.*, X-ray diffraction results from Mars Science Laboratory: Mineralogy of Rocknest at Gale crater. *Science* **341**, 1238932 (2013).
- D. F. Blake *et al.*, Curiosity at Gale crater, Mars: Characterization and analysis of the Rocknest sand shadow. *Science* **341**, 1239505 (2013).
- L. A. Leshin *et al.*, Volatile, isotope, and organic analysis of martian fines with the Mars Curiosity rover. *Science* **341**, 1238937 (2013).
- The RMSEP on the sum of total oxides is ~11.5 wt %.
- S. Clegg *et al.*, High calcium phase observations at Rocknest with ChemCam. *Lunar Planet. Sci. Conf.* **44**, abstract 2087 (2013).

50. M. Fisk *et al.*, Missing components in chemical profiles of a sand drift in Gale crater. *Lunar Planet. Sci. Conf.* **44**, abstract 2156 (2013).
51. N. Rennó *et al.*, Possible physical and thermodynamical evidence for liquid water at the Phoenix landing site. *J. Geophys. Res.* **114**, E00E03 (2009). doi: [10.1029/2009JE003362](https://doi.org/10.1029/2009JE003362)
52. Although direct quantitative estimates of H abundance by ChemCam require laboratory experiments that have not been performed, we discuss in the supplementary material some preliminary estimates of upper limits of the observations reported here based on quantitative results by SAM. These estimates thus also rely on SAM uncertainties.
53. H. E. Newsom *et al.*, Geochemistry of Martian soil and bedrock in mantled and less mantled terrains with gamma ray data from Mars Odyssey. *J. Geophys. Res.* **112**, E3S012 (2007). doi: [10.1029/2006JE002791](https://doi.org/10.1029/2006JE002791)
54. R. V. Morris *et al.*, Mössbauer mineralogy of rock, soil, and dust at Meridiani Planum, Mars: Opportunity's journey across sulfate-rich outcrop, basaltic sand and dust, and hematite lag deposits. *J. Geophys. Res.* **111**, E12S15 (2006). doi: [10.1029/2006JE002791](https://doi.org/10.1029/2006JE002791)
55. R. V. Morris *et al.*, Mössbauer mineralogy of rock, soil, and dust at Gusev Crater, Mars: Spirit's journey through weakly altered olivine basalt on the plains and pervasively altered basalt in the Columbia Hills. *J. Geophys. Res.* **111**, E12S15 (2006). doi: [10.1029/2006JE002791](https://doi.org/10.1029/2006JE002791)
56. W. Goetz *et al.*, Indication of drier periods on Mars from the chemistry and mineralogy of atmospheric dust. *Nature* **436**, 62–65 (2005). doi: [10.1038/nature03807](https://doi.org/10.1038/nature03807); pmid: [16001062](https://pubmed.ncbi.nlm.nih.gov/16001062/)
57. W. T. Pike *et al.*, Quantification of the dry history of the Martian soil inferred from in situ microscopy. *Geophys. Res. Lett.* **38**, L24201 (2011). doi: [10.1029/2011GL049896](https://doi.org/10.1029/2011GL049896)
58. R. A. Eggleton, D. B. Tilley, Hisingerite: A ferric kaolin mineral with curved morphology. *Clays Clay Miner.* **46**, 400–413 (1998). doi: [10.1346/CCMN.1998.0460404](https://doi.org/10.1346/CCMN.1998.0460404)
59. E. B. Rampe *et al.*, Allophane detection on Mars with Thermal Emission Spectrometer data and implications for regional-scale chemical weathering processes. *Geology* **40**, 995–998 (2012). doi: [10.1130/G33215.1](https://doi.org/10.1130/G33215.1)
60. S. Le Mouéléc *et al.*, An iterative least squares approach to decorrelate minerals and ices contributions in hyperspectral images: Application to Cuprite (Earth) and Mars, First Workshop on Hyperspectral Image and Signal Processing: Evolution in Remote Sensing, 2009. WHISPERS, 26–28 Aug. 2009 (2009). doi: [10.1109/WHISPERS.2009.5289003](https://doi.org/10.1109/WHISPERS.2009.5289003)
61. S. Shoji, N. Masami, R. Dahlgren, *Volcanic Ash Soils: Genesis, Properties and Utilization* (Elsevier, Amsterdam, 1993).
62. Soil Survey Staff, *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys* (Natural Resources Conservation Service, U.S. Department of Agriculture, ed. 2, 1999).
63. B. M. Jakosky, The seasonal cycle of water on Mars. *Space Sci. Rev.* **41**, 131–200 (1985). doi: [10.1007/BF00241348](https://doi.org/10.1007/BF00241348)
64. B. M. Jakosky, R. M. Haberle, The seasonal behavior of water on Mars. In *Mars*, H. H. Kieffer, B. M. Jakosky, C. Snyder, M. S. Matthews, Eds. (Univ. of Arizona Press, Tucson, 1992), pp. 969–1016.
65. F. Bartoli, A. J. Poulenard, B. E. Schouller, Influence of allophane and organic matter contents on surface properties of Andosols. *Eur. J. Soil Sci.* **58**, 450–464 (2007). doi: [10.1111/j.1365-2389.2007.00899.x](https://doi.org/10.1111/j.1365-2389.2007.00899.x)
66. H. Khan *et al.*, Adsorption of water on nano-ball allophane. *Clay Sci.* **12** (suppl. 2), 261–266 (2006).
67. A. Pommerol, B. Schmitt, P. Beck, O. Brissaud, Water sorption on Martian regolith analogs: Thermodynamics and near-infrared reflectance spectroscopy. *Icarus* **204**, 114–136 (2009). doi: [10.1016/j.icarus.2009.06.013](https://doi.org/10.1016/j.icarus.2009.06.013)
68. J. Jänchen, R. V. Morris, D. L. Bish, M. Janssen, U. Hellwig, The H₂O and CO₂ adsorption properties of phyllosilicate-poor palagonitic dust and smectites under Martian environmental conditions. *Icarus* **200**, 463–467 (2009). doi: [10.1016/j.icarus.2008.12.006](https://doi.org/10.1016/j.icarus.2008.12.006)
69. P. Beck, A. Pommerol, B. Schmitt, O. Brissaud, Kinetics of water adsorption on minerals and the breathing of the Martian regolith. *J. Geophys. Res.* **115**, E10011 (2010). doi: [10.1029/2009JE003539](https://doi.org/10.1029/2009JE003539)
70. Y. Kitagawa, Dehydration of allophane and its structural formula. *Am. Mineral.* **59**, 1094–1098 (1974).
71. L. Maltagliati *et al.*, Annual survey of water vapor behavior from the OMEGA mapping spectrometer onboard Mars Express. *Icarus* **213**, 480–495 (2011). doi: [10.1016/j.icarus.2011.03.030](https://doi.org/10.1016/j.icarus.2011.03.030)
72. E. V. Ballou, P. C. Wood, T. Wydeven, M. E. Lehwalt, R. E. Mack, Chemical interpretation of Viking Lander 1 life detection experiment. *Nature* **271**, 644–645 (1978). doi: [10.1038/271644a0](https://doi.org/10.1038/271644a0)
73. D. M. Anderson, A. R. Tice, The analysis of water in the Martian regolith. *J. Mol. Evol.* **14**, 33–38 (1979). doi: [10.1007/BF01732365](https://doi.org/10.1007/BF01732365); pmid: [522156](https://pubmed.ncbi.nlm.nih.gov/522156/)

Acknowledgments: This research was carried out with funding from the Centre National d'Etudes Spatiales (CNES). Work in the United States was carried out under contract from NASA's Mars Program Office. W.G. acknowledges partial funding from Deutsche Forschungsgemeinschaft grant GO 2288/1-1. This team gratefully acknowledges JPL for developing and leading this successful mission. The data reported in this paper are archived at the Planetary Data System, accessible at <http://pds-geosciences.wustl.edu/missions/msl/index.htm>.

Supplementary Materials

www.sciencemag.org/content/341/6153/1238670/suppl/DC1
MSL Science Team Author List
Supplementary Text
Figs. S1 to S4
References (74–89)

3 April 2013; accepted 15 August 2013
10.1126/science.1238670

X-ray Diffraction Results from Mars Science Laboratory: Mineralogy of Rocknest at Gale Crater

D. L. Bish,^{1*} D. F. Blake,² D. T. Vaniman,³ S. J. Chipera,⁴ R. V. Morris,⁵ D. W. Ming,⁵ A. H. Treiman,⁶ P. Sarrazin,⁷ S. M. Morrison,⁸ R. T. Downs,⁸ C. N. Achilles,⁹ A. S. Yen,¹⁰ T. F. Bristow,² J. A. Crisp,¹⁰ J. M. Morookian,¹⁰ J. D. Farmer,¹¹ E. B. Rampe,⁵ E. M. Stolper,¹² N. Spanovich,¹⁰ MSL Science Team†

The Mars Science Laboratory rover Curiosity scooped samples of soil from the Rocknest aeolian bedform in Gale crater. Analysis of the soil with the Chemistry and Mineralogy (CheMin) x-ray diffraction (XRD) instrument revealed plagioclase (~An57), forsteritic olivine (~Fo62), augite, and pigeonite, with minor K-feldspar, magnetite, quartz, anhydrite, hematite, and ilmenite. The minor phases are present at, or near, detection limits. The soil also contains 27 ± 14 weight percent x-ray amorphous material, likely containing multiple Fe³⁺- and volatile-bearing phases, including possibly a substance resembling hisingerite. The crystalline component is similar to the normative mineralogy of certain basaltic rocks from Gusev crater on Mars and of martian basaltic meteorites. The amorphous component is similar to that found on Earth in places such as soils on the Mauna Kea volcano, Hawaii.

Numerous observations of the martian surface, both in situ and from orbit, suggest that basaltic soil across the planet has a fairly uniform chemical composition. Global-scale aeolian mixing of the finest grains is a major factor in this uniformity, but not too disparate basaltic compositions across the planet may also be a contributing factor (1, 2). High-quality chemical data for martian soils are available from the Pathfinder, Mars Exploration Rover (MER), and Phoenix missions (2–4), and phase information has been provided through MER thermal emission and Mössbauer spectroscopic measurements (3, 5–9). The Chemistry and Mineralogy (CheMin) instrument onboard the Mars Science Laboratory (MSL) rover Curiosity uses x-ray diffraction (XRD), which is generally the preferred and the most definitive method for determining the nature of crystalline phases (such as minerals) in solid samples. CheMin's XRD analysis on Mars coincided with the 100th-year anniversary of the discovery of XRD by von Laue (10).

On the basis of Alpha Particle X-ray Spectrometer (APXS) chemical analyses, the Rocknest aeolian bedform is considered to be representative of global basaltic soil at Gale crater (11–13). Curiosity delivered the <150-μm-size fraction of three samples of loose, unconsolidated material ("soil") acquired at Rocknest to the CheMin instrument inside the body of the rover, and CheMin measured two-dimensional (2D) diffraction data (Fig. 1, scoop five) for the three samples (details are available in materials and methods). Imaging shows that the soil has a range of particle sizes, 1 to 2 mm and smaller, presumably representing contributions from global, regional, and local sources (14). The larger particles at the top of the bedform appear to be armoring the bedform. The term soil is used here to denote any loose, unconsolidated materials that can be distinguished from rocks, bedrock, or strongly cohesive sediments. No implication of the presence or absence of organic materials or living matter is intended, nor is the genesis of the deposit inferred.

Results

Crystalline Components

Initial analyses of the measured diffraction data from three different scoops revealed the presence of plagioclase feldspar, forsteritic olivine, augite, and another pyroxene, with no evidence of any phyllosilicate mineral. Rietveld refinements including numerous candidate phases revealed the presence of pigeonite with augite. Refinements using augite with orthopyroxene or clinopyroxene were inferior to an augite-pigeonite model. A single-plagioclase model was as good as a model with two plagioclases of different composition

(such as different unit-cell parameters or Na-Ca site occupancies), so only a single plagioclase was used. However, we cannot exclude the presence of multiple or zoned pyroxene, olivine, and plagioclase compositions. Refinements also clarified the minor mineral species and their abundances and allowed exclusion of many possible minerals. The presence of minor phases was evaluated individually by including each in the model and evaluating their effect on the fit (Fig. 2). All three scoop samples produced similar results, although changes in the XRD pattern for scoop three as a function of time suggested that sample was ejected from the XRD sample cell by vibration, and these data were not used. The 2σ values given in Table 1 are from the Rietveld refinement; they show that several minor phases are questionable, with errors close to or exceeding the refined values. Refined unit-cell parameters for the major phases (Table 2) were used to estimate the compositions of these phases by comparison with literature values (15). Such comparisons gave $(\text{Mg}_{0.62(3)}\text{Fe}_{0.38})_2\text{SiO}_4$ for the composition of the olivine mineral (parenthetical numbers in the subscripts refer to standard errors from fits of our unit-cell parameters to literature values and do not consider the errors on refined unit-cell parameters). This composition agrees well with the refined Mg-Fe site occupancies, $(\text{Mg}_{0.64(3)}\text{Fe}_{0.36})_2\text{SiO}_4$. The plagioclase unit-cell parameters gave a composition of $(\text{Ca}_{0.57(13)}\text{Na}_{0.43})(\text{Al}_{1.57}\text{Si}_{2.43})\text{O}_8$, which does not agree with the refined site-occupancy data (close to the Na end-member composition). We consider the unit-cell parameter trends to be more reliable than the site occupancy information, which is based on diffracted intensities over the short angular range (<55° 2θ) provided by CheMin. In addition, diffraction intensities may have been affected by preferred crystallite orientation. Although we did not use a preferred orientation correction in our

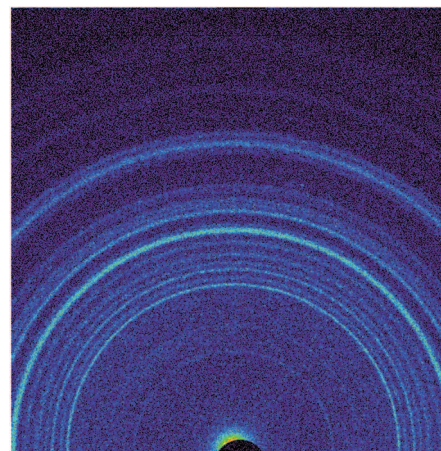


Fig. 1. CheMin 2D XRD pattern of scoop 5, representing 26.9 hours of integration time. Image contrast has been enhanced and colorized to emphasize the Debye diffraction rings. The black semicircle at the bottom is the shadow of the beam stop.

¹Department of Geological Sciences, Indiana University, Bloomington, IN 47405, USA. ²NASA Ames Research Center, Moffett Field, CA 94035, USA. ³Planetary Science Institute, Tucson, AZ 85719, USA. ⁴Chesapeake Energy, Oklahoma City, OK 73154, USA. ⁵NASA Johnson Space Center, Houston, TX 77058, USA. ⁶Lunar and Planetary Institute, Houston, TX 77058, USA. ⁷In-Xitu, Campbell, CA 95008, USA. ⁸Department of Geology, University of Arizona, Tucson, AZ 85721, USA. ⁹Engineering and Science Contract Group/United Technologies Corporation Aerospace Systems, Houston, TX 77058, USA. ¹⁰Jet Propulsion Laboratory/California Institute of Technology, Pasadena, CA 91109, USA. ¹¹Department of Geological Sciences, Arizona State University, Tempe, AZ 85287, USA. ¹²California Institute of Technology, Pasadena, CA 91125, USA.

*Corresponding author. E-mail: bish@indiana.edu

†MSL Science Team authors and affiliations are listed in the supplementary materials.

Fig. 2. Rietveld refinement results for scoop 5 (final $R_{wp} = 4.3\%$; R_{wp} = weighted profile residual from the Rietveld refinement). Observed (blue) versus calculated (red) pattern, with difference curve (obs-calc) at the bottom (gray). The difference peak at $\sim 25.6^\circ 2\theta$ is due to scattering from the aluminum light shield. Minerals included in the refinement model are listed in Table 1.

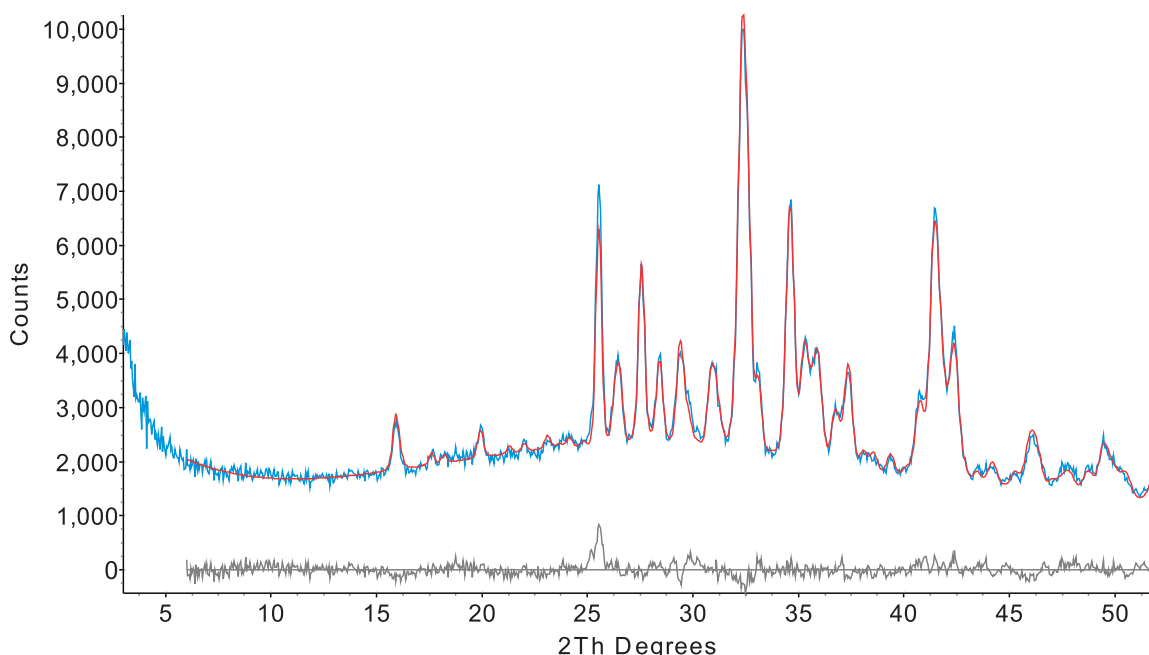


Table 1. Crystalline components (amorphous-free, normalized to 100%) of the Rocknest scoop 5 soil.

Mineral	Weight (%)	2 σ (%)
Plagioclase (~An57)	40.8	2.4
Forsterite (~Fo62)	22.4	1.9
Augite	14.6	2.8
Pigeonite	13.8	2.8
Magnetite	2.1	0.8
Anhydrite	1.5	0.7
Quartz	1.4	0.6
Sanidine*	1.3	1.3
Hematite*	1.1	0.9
Ilmenite*	0.9	0.9

*At or near detection limit

final refinements, we did see indications of minor orientation effects in the data, as evidenced by small improvements in Rietveld fits when a preferred orientation correction was used for plagioclase. Refined unit-cell parameters for pigeonite gave a composition of $(\text{Mg}_{1.13}\text{Fe}_{0.68}\text{Ca}_{0.19})\text{Si}_2\text{O}_6$, compared with octahedral site-occupancy refinement results of $(\text{Mg}_{1.71(15)}\text{Fe}_{0.13}\text{Ca}_{0.16})\text{Si}_2\text{O}_6$. Refined unit-cell parameters for augite gave a composition of $(\text{Mg}_{0.88(10)}\text{Fe}_{0.37}\text{Ca}_{0.75(4)})\text{Si}_2\text{O}_6$, which is broadly consistent with octahedral site-occupancy refinement results of $(\text{Mg}_{0.58(15)}\text{Fe}_{0.71}\text{Ca}_{0.71})\text{Si}_2\text{O}_6$. Unit-cell parameters suggest atomic Mg:Fe ratios for augite and pigeonite of 2.4 and 1.7, respectively. Comparable ratios imply the two pyroxenes originated from the same magma, rather than having experienced post-crystallization alteration (such as pigeonite inversion) or being from different source regions (16).

We also evaluated the presence of a variety of Ca-, K-, Fe- and Mg-sulfates; halides; Ca-, Mg-, and Fe-carbonates; phosphates; and Ca- and Mg-

perchlorates (many of these hydrated). It is straightforward to include each of these phases in the Rietveld model and evaluate its potential contribution to the diffraction pattern, both visually and based on the fit parameter (R_{wp}). Using this approach, we found no evidence for the presence of any of these phases, other than those listed in Table 1, several of which have 2σ uncertainties greater than the refined abundances. If halides, perchlorates, carbonates, phosphates, or other sulfate phases are present as crystalline phases, they are below the detection limits of the CheMin instrument [1 to 2 weight percent (wt %)]. Although data from the Sample Analysis at Mars (SAM) instrument suite suggest the presence of a small amount of perchlorate (0.3 to 0.5 wt %) in the Rocknest soil (17, 18), models that included Mg-perchlorate-6H₂O and Ca-perchlorate-4H₂O refined to 0 wt % for these phases; thus, we find no diffraction evidence for either of these phases.

Amorphous or Poorly Ordered Components

The elevated background in the 15 to 40° 2θ range in Fig. 2 results from the presence of one or more amorphous or poorly ordered components. The low-angle background is also elevated significantly above that seen with empty cells. Hence, we also analyzed the CheMin XRD data using a modified version of the FULLPAT program (19), which allows direct determination of the abundance of amorphous components. The FULLPAT analyses explicitly used patterns for both ordered and amorphous phases, and the entire diffraction patterns, including background, were fit. The abundances of crystalline and amorphous phases (Table 3) were normalized to sum to 100 wt % in accordance with the adiabatic method (20), and scoops four and five gave an average amorphous content of 27 wt %. The uncertainty on individual amorphous values may be as high as

50 wt % relative. Of the amorphous standards included in the analysis, only one allophane and a basaltic glass gave positive concentrations. The materials used as standards for amorphous materials were chosen as reasonable representatives of expected materials in the Mars aeolian bedform. However, the exact nature of the amorphous component remains unclear. Although a synthetic SO₃- and Cl-free Gusev-composition basaltic glass was the dominant amorphous component in our FULLPAT fit, it may be just one of many amorphous components that have similar XRD patterns, and these analyses do not unambiguously identify the amorphous component (or components). Similarly, allophane may be an XRD surrogate for another amorphous phase such as the Fe³⁺-bearing phase hisingerite, and the sample may contain a small amount of such material.

The Rocknest soil contains glassy-luster spherules that may have an impact or pyroclastic origin (21), but our results do not necessarily indicate that the amorphous component in Rocknest soil is dominated by basaltic glass; other observations suggest otherwise. MER Mössbauer analyses of basaltic soils from Gusev and Meridiani show substantial abundances of an amorphous phase containing Fe³⁺ [generically referred to as nanophase ferric oxide (npOx)] (7), and taken together, the MSL CheMin and SAM data suggest that the Gusev amorphous phase (or phases) is volatile rich (containing, for example, H₂O/OH or SO₃) (18). The abundances (Table 1) and chemistry (from unit-cell parameters) of the crystalline phases identified by CheMin, coupled with mass-balance considerations from APXS analyses of Rocknest soil, suggest that the amorphous component is SO₃- and Cl-bearing (11, 22). Last, if MER-like levels of Fe³⁺ are present in Rocknest soil, then the amorphous component must also be the carrier of the Fe³⁺ (with the exception of

Table 2. Refined unit-cell parameters for major crystalline phases in the Rocknest soil (scoop five).

Mineral	Unit-cell parameter	Value (ESD)
Forsterite	<i>a</i> (Å)	10.327 (7)
	<i>b</i> (Å)	6.034 (7)
	<i>c</i> (Å)	4.771 (5)
Plagioclase	<i>a</i> (Å)	8.177 (6)
	<i>b</i> (Å)	12.868 (9)
	<i>c</i> (Å)	7.113 (5)
	α (degrees)	93.43 (4)
	β (degrees)	116.26 (2)
Pigeonite	γ (degrees)	90.13 (3)
	<i>a</i> (Å)	9.652 (9)
	<i>b</i> (Å)	8.92 (1)
	<i>c</i> (Å)	5.254 (7)
	β (degrees)	108.0 (1)
Augite	<i>a</i> (Å)	9.782 (9)
	<i>b</i> (Å)	8.939 (9)
	<i>c</i> (Å)	5.269 (7)
	β (degrees)	106.25 (9)

Table 3. Amorphous contents (wt %) from FULLPAT analyses of scoops 4 and 5.

	Scoop 4	Scoop 5
Gusev-composition		
basaltic glass	23	25
Allophane-like material	3	2
Total	26	27

magnetite and possible hematite) that is responsible for the reddish color of the soil (14, 22). We did not have access to a pure sample of npOx for use as a standard in FULLPAT analyses and cannot exclude this from the amorphous phase inventory in these samples.

Discussion

Our XRD data reveal a rich inventory of crystalline and amorphous phases in Rocknest soil and provide insight into chemical and physical weathering processes on Mars. The crystalline component is dominated by plagioclase, olivine, augite, and pigeonite, which is consistent with and confirms a variety of previous orbital and lander analyses (2, 3, 5–9). This assemblage, particularly the Fe-rich forsterite and the presence of a substantial amorphous component, is consistent with limited aqueous alteration, similar to conclusions from the Phoenix lander (23). We found no XRD evidence for zeolite minerals, which were previously proposed (24) as an alternative to feldspar in martian dust. CheMin is sensitive to the presence of zeolite minerals because their major diffraction peaks lie in an angular range with few or no other peaks. The presence of pigeonite and the similarity of the augite and pigeonite Fe:Mg ratios imply that the crystalline component results from the near-surface crystallization of basaltic lavas as opposed to formation in plutonic rocks. The crystalline component is

very similar to normative basalt mineralogies calculated from Gusev APXS data (25) and is also qualitatively similar to mineralogies of martian basaltic meteorites (16). Although hydrous phyllosilicates (such as smectites) have been identified on the martian surface through orbital spectral data, the XRD data did not reveal any phyllosilicate in this soil. The absence of smectites is somewhat surprising because orbital spectral data suggest the presence of smectites in and around Gale crater (26). Because diffraction peaks from typical phyllosilicates (such as smectites) generally are quite broad, our detection limits for such minerals are comparatively poor, probably on the order of at least 5%. The lack of any detectable hydrated crystalline phase is important, as is the lack of detectable crystalline sulfate (other than minor anhydrite), perchlorate, or chloride phases. This result, coupled with the observation with the SAM instrument of volatile evolution (18), implies that virtually the entire volatile inventory of the Rocknest soil is associated with the amorphous component, an important detail that previous investigations were unable to detect. By combining these quantitative XRD results with compositional estimates from unit-cell parameters and bulk chemistry, it has been possible to determine the chemical compositions of the major phases, including that of the amorphous component (or components) (11). With the exception of the S content, the amorphous component (or components) are remarkably similar to those found on Earth in volcanic soils, such as those on the flanks of Mauna Kea volcano, Hawaii (27). The CheMin XRD results should be applicable to previous and future soil measurements on Mars because soil compositions from many different measurements at several locales appear so similar (28). In addition, these data provide critical ground-truth information on martian soils and expand our understanding of the fine-grained component on the martian surface.

Materials and Methods

Samples and Measurements

Scoops three, four, and five of the soil were introduced into the Collection and Handling for In situ Martian Rock Analysis (CHIMRA) sample processing system on *Curiosity*. Each scoop sample was passed through a 150- μ m sieve (thus excluding the coarser-grained material visible in images of the bedform) before delivering a portion to the CheMin inlet funnel. Scoops one and two were used to clean the CHIMRA system and were not introduced into *Curiosity*'s instruments.

Scoops three and four were placed into a sample cell with 10- μ m-thick Kapton (Dupont, Wilmington, Delaware) windows, and scoop five was placed into a cell with 6- μ m-thick Mylar (Dupont, Wilmington, Delaware) windows. Both types of cells have the potential to contribute broad scattering signatures to the diffraction patterns.

In addition, an aluminized light shield also contributes “peaks” to the observed diffraction patterns. Kapton contributes a broad peak centered at ~ 15 Å, whereas Mylar has a very small amount of scattering at low angles. Mylar cells are therefore preferred when searching for diffraction signatures from phyllosilicates or other materials having diffraction peaks at low angles. Only ~ 10 mm³ of material is required to fill the active portion of the sample cell, which is a disc-shaped volume 8 mm in diameter and 175 μ m thick. The collimated ~ 70 μ m diameter x-ray beam illuminates the center of the sample cell. A piezoelectric vibration system on each cell shakes the material during analysis, causing all of the grains in the cell to pass through the x-ray beam in random orientations over the time course of an analysis.

CheMin measures XRD and x-ray fluorescence (XRF) data simultaneously using Co radiation in transmission geometry (29). The instrument operates in single-photon counting mode so that the majority of CCD pixels are struck between each readout by either a single x-ray photon or by no photons. In this way, the system can determine both the energy of the photons striking the CCD (XRF) and the 2D position of each photon (XRD) (29). The energy and positional information of detected photons in each frame are summed over repeated 10-s measurements into a “minor frame” of 30 min of data (180 frames). CheMin collects as many minor frames as possible for the available analysis time, which is usually five to seven per night, and these are summed to create one data file for each night of data collection. The 2D distribution of Co K α x-ray intensity represents the XRD pattern of the sample (Fig. 1), and circumferential integration of these rings, corrected for arc length, produces a conventional 1D XRD pattern. CheMin generally operates for only a few hours each night during which time the CCD is at its lowest temperature. Thus, XRD data were acquired over multiple nights for each scoop sample to provide acceptable counting statistics. Data were measured for 3.8 hours (scoop 3), 15.7 hours (scoop 4), and 26.9 hours (scoop 5). The data for individual minor frames and for each night's analyses were examined separately, and there was no evidence of any changes in instrumental parameters as a function of time over the duration of these analyses. Before analysis of each new scoop, measurements were made of the empty cell to confirm that it was indeed empty before receiving the sample. We calibrated the flight instrument on the ground before flight using a quartz-beryl standard, and measurement of this standard on Mars showed no changes in instrument geometry or dimensions.

Crystalline Components

All XRD data were first evaluated by comparisons and searches of the International Centre for Diffraction Data (ICDD) Powder Diffraction File using Bruker AXS DIFFRAC.EVA (Bruker AXS, Karlsruhe, Germany, 2000) and MDI Jade (Materials Data Incorporated, Livermore, California)

software packages, which revealed the presence of plagioclase feldspar, forsteritic olivine, augite, and another pyroxene. There was no evidence of any phyllosilicate, which would have produced diffraction peaks at low angles (5 to 15° 2θ). The comparatively large instrumental peak widths for the CheMin instrument ($\sim 0.3^\circ$ 2θ full-width at half-maximum at 25° 2θ) limited our ability to determine accurately the presence of minor crystalline phases (<3 wt %). The data were analyzed further via Rietveld methods, using Topas (Bruker AXS, 2000). We used the fundamental-parameters approach within Topas, along with additional convolutions, to model the experimental profiles. We also used an emission spectrum including Co K α , with a refinable Co K β component. The Rietveld method involves constructing a model consisting of the crystal structures of all component phases, and the differences between the observed and simulated diffraction patterns are minimized by varying components of the model, including scale factors (related to phase abundance), unit-cell parameters, and crystallite-size and strain broadening parameters for each phase. Atomic positions and site occupancies were generally not varied, although octahedral site occupancies were varied for forsteritic olivine, augite, and pigeonite, and Na-Ca occupancies were varied for the plagioclase component. This method thus provides information on all well-ordered phases (crystalline phases), but it is not directly applicable to disordered phases such as clay minerals or amorphous components.

Amorphous or Poorly Ordered Components

FULLPAT operates on the principle that diffraction and scattering patterns for all phases in a sample are additive. By fitting full diffraction patterns—including the background, which contains important information on sample composition and matrix effects—explicit analysis of amorphous or partially ordered materials can often be readily accomplished if the amorphous/disordered phases are included in the analyses as distinct phases. Thus, FULLPAT allows direct analysis of the abundance of amorphous components, rather than determining them as the difference from 100 wt % in an internal standard quantitative analysis. Like all full-pattern fitting methods, accurate analysis requires representative standards or structure models. A large variety of pure mineral standards, disordered materials (allophanes, ferrihydrite, and aluminosilicate gels), and a synthetic basaltic glass of Gusev composition were measured. Each of these was run as a pure phase and was also mixed with a beryl standard in a 50:50 weight ratio to determine a reference intensity ratio (RIR) for subsequent use in FULLPAT (30, 31). All standard data were measured on a CheMin IV instrument at the NASA Johnson Space Center; the CheMin IV instrument geometry is very similar to the instrument on MSL and is considered a good proxy for the flight instrument. Peak areas for each

phase were compared against the intensity of the beryl 100 reflection, and the measured beryl RIR of 1.70 relative to corundum (measured on a laboratory instrument) was used to convert the RIR(beryl) to the conventional RIR(corundum) value. During FULLPAT analysis, the intensity of each standard pattern was normalized to the intensity of a pure pattern of corundum used as datum. Thus, using this corundum datum 113 reflection intensity and the measured RIR for each standard phase, the pattern of each disordered phase could be normalized to the appropriate overall intensity based on its measured intensity area used for RIR determination.

Because few standard data for pure phases have been measured on the CheMin flight instrument, an alternate method for calculating standard data representative of the MSL CheMin instrument was often used. This process involved first determining instrumental peak shapes and widths as function of 2θ by using the beryl standard measured on the MSL instrument. We then calculated diffraction patterns for each standard using the appropriate crystal structure information and the instrumental profiles determined above for Co K α radiation. The final step in calculation of standard data for FULLPAT was to normalize the intensity of the calculated pattern to the corundum datum pattern by using the calculated RIR as outlined above. The scaled measured and calculated library patterns, for both ordered and amorphous phases, were then used with FULLPAT.

References and Notes

1. B. C. Clark *et al.*, Chemical composition of martian fines. *J. Geophys. Res.* **87**, 10059–10067 (1982). doi: [10.1029/JB087iB12p10059](https://doi.org/10.1029/JB087iB12p10059)
2. R. Gellert *et al.*, Chemistry of rocks and soils in Gusev Crater from the alpha particle x-ray spectrometer. *Science* **305**, 829–832 (2004). doi: [10.1126/science.1099913](https://doi.org/10.1126/science.1099913); pmid: [15297665](https://pubmed.ncbi.nlm.nih.gov/15297665/)
3. A. S. Yen *et al.*, An integrated view of the chemistry and mineralogy of martian soils. *Nature* **436**, 49–54 (2005). doi: [10.1038/nature03637](https://doi.org/10.1038/nature03637); pmid: [16001059](https://pubmed.ncbi.nlm.nih.gov/16001059/)
4. M. H. Hecht *et al.*, Detection of perchlorate and the soluble chemistry of martian soil at the Phoenix lander site. *Science* **325**, 64–67 (2009). pmid: [19574385](https://pubmed.ncbi.nlm.nih.gov/19574385/)
5. I. O. McGlynn, C. M. Fedo, H. Y. McSweeney Jr., Soil mineralogy at the Mars Exploration Rover landing sites: An assessment of the competing roles of physical sorting and chemical weathering. *J. Geophys. Res.* **117**, E01006 (2012). doi: [10.1029/2011JE003861](https://doi.org/10.1029/2011JE003861)
6. H. Y. McSweeney Jr., I. O. McGlynn, A. D. Rogers, Determining the modal mineralogy of Martian soils. *J. Geophys. Res.* **115**, E00F12 (2010). doi: [10.1029/2010JE003582](https://doi.org/10.1029/2010JE003582)
7. R. V. Morris *et al.*, Mössbauer mineralogy of rock, soil, and dust at Gusev Crater, Mars: Spirit's journey through weakly altered olivine basalt on the Plains and pervasively altered basalt in the Columbia Hills. *J. Geophys. Res.* **111**, E02513 (2006). doi: [10.1029/2005JE002584](https://doi.org/10.1029/2005JE002584)
8. R. V. Morris *et al.*, Mineralogy at Gusev crater from the Mossbauer spectrometer on the Spirit rover. *Science* **305**, 833–836 (2004). doi: [10.1126/science.1100020](https://doi.org/10.1126/science.1100020); pmid: [15297666](https://pubmed.ncbi.nlm.nih.gov/15297666/)
9. P. R. Christensen *et al.*, Mineralogy at Meridiani Planum from the Mini-TES Experiment on the Opportunity Rover. *Science* **306**, 1733–1739 (2004). doi: [10.1126/science.1104909](https://doi.org/10.1126/science.1104909); pmid: [15576609](https://pubmed.ncbi.nlm.nih.gov/15576609/)
10. M. Eckert, Max von Laue and the discovery of x-ray diffraction in 1912. *Ann. Phys.* **524**, A83–A85 (2012). doi: [10.1002/andp.201200724](https://doi.org/10.1002/andp.201200724)
11. R. V. Morris *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1653; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
12. A. S. Yen *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 2495; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
13. R. Gellert *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1432; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
14. D. F. Blake *et al.*, Curiosity at Gale crater, Mars: Characterization and analysis of the Rocknest sand shadow. *Science* **341**, 1239505 (2013).
15. S. M. Morrison *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1831; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
16. A. H. Treiman *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1113; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
17. P. D. Archer Jr. *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 2168; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
18. L. A. Leshin *et al.*, Volatile, isotope, and organic analysis of martian fines with the Mars Curiosity rover. *Science* **341**, 1238937 (2013).
19. S. J. Chipera, D. L. Bish, FULLPAT: A full-pattern quantitative analysis program for x-ray powder diffraction using measured and calculated patterns. *J. Appl. Cryst.* **35**, 744–749 (2002). doi: [10.1107/S0021889802017405](https://doi.org/10.1107/S0021889802017405)
20. F. H. Chung, Quantitative interpretation of x-ray diffraction patterns of mixtures. II. Adiabatic principle of x-ray diffraction analysis of mixtures. *J. Appl. Cryst.* **7**, 526–531 (1974). doi: [10.1107/S0021889874010387](https://doi.org/10.1107/S0021889874010387)
21. R. Yingst *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1257; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
22. D. F. Blake *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1289; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).
23. W. T. Pike *et al.*, Quantification of the dry history of the Martian soil inferred from in situ microscopy. *Geophys. Res. Lett.* **38**, L24201 (2011). doi: [10.1029/2011GL049896](https://doi.org/10.1029/2011GL049896)
24. S. W. Ruff, Spectral evidence for zeolite in the dust on Mars. *Icarus* **168**, 131–143 (2004). doi: [10.1016/j.icarus.2003.11.003](https://doi.org/10.1016/j.icarus.2003.11.003)
25. D. W. Ming *et al.*, Geochemical and mineralogical indicators for aqueous processes in the Columbia Hills of Gusev crater, Mars. *J. Geophys. Res. Planets* **111**, E02512 (2006). doi: [10.1029/2005JE002560](https://doi.org/10.1029/2005JE002560)
26. R. E. Milliken, J. P. Grotzinger, B. J. Thomson, Paleoclimate of Mars as captured by the stratigraphic record in Gale Crater. *Geophys. Res. Lett.* **37**, L04201 (2010). doi: [10.1029/2009GL041870](https://doi.org/10.1029/2009GL041870)
27. R. V. Morris *et al.*, Phyllosilicate-poor palagonitic dust from Mauna Kea Volcano (Hawaii): A mineralogical analogue for magnetic martian dust? *J. Geophys. Res.* **106**, 5057–5083 (2001). doi: [10.1029/2000JE001328](https://doi.org/10.1029/2000JE001328)
28. R. Gellert *et al.*, Alpha Particle X-Ray Spectrometer (APXS): Results from Gusev crater and calibration report. *J. Geophys. Res.* **111**, E02S05 (2006). doi: [10.1029/2005JE002555](https://doi.org/10.1029/2005JE002555)
29. D. Blake *et al.*, Characterization and calibration of the CheMin mineralogical instrument on Mars Science Laboratory. *Space Sci. Rev.* **170**, 341–399 (2012). doi: [10.1007/s11214-012-9905-1](https://doi.org/10.1007/s11214-012-9905-1)
30. C. N. Achilles, R. V. Morris, S. J. Chipera, D. W. Ming, E. B. Rampe, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 3072; published

- on CD by the Lunar and Planetary Institute, Houston, TX (2013).
31. E. B. Rampe *et al.*, presented at the 44th Lunar and Planetary Science Conference, March 2013, abstract 1188; published on CD by the Lunar and Planetary Institute, Houston, TX (2013).

Acknowledgments: Support from the NASA Mars Science Laboratory Mission is gratefully acknowledged. Some of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. XRD data presented here are archived in the Planetary Data System (PDS, pds.nasa.gov).

Supplementary Materials

www.sciencemag.org/content/341/6153/1238932/suppl/DC1

MSL Science Team Author List

9 April 2013; accepted 1 August 2013
10.1126/science.1238932

Curiosity at Gale Crater, Mars: Characterization and Analysis of the Rocknest Sand Shadow

D. F. Blake,^{1*} R. V. Morris,² G. Kocurek,³ S. M. Morrison,⁴ R. T. Downs,⁴ D. Bish,⁵ D. W. Ming,² K. S. Edgett,⁶ D. Rubin,^{7†} W. Goetz,⁸ M. B. Madsen,⁹ R. Sullivan,¹⁰ R. Gellert,¹¹ I. Campbell,¹¹ A. H. Treiman,¹² S. M. McLennan,¹³ A. S. Yen,¹⁴ J. Grotzinger,¹⁵ D. T. Vaniman,¹⁶ S. J. Chipera,¹⁷ C. N. Achilles,² E. B. Rampe,² D. Sumner,¹⁸ P.-Y. Meslin,¹⁹ S. Maurice,¹⁹ O. Forni,¹⁹ O. Gasnault,¹⁹ M. Fisk,²⁰ M. Schmidt,²¹ P. Mahaffy,²² L. A. Leshin,²³ D. Glavin,²² A. Steele,²⁴ C. Freissinet,²² R. Navarro-González,²⁵ R. A. Yingst,¹⁶ L. C. Kah,²⁶ N. Bridges,²⁷ K. W. Lewis,²⁸ T. F. Bristow,¹ J. D. Farmer,²⁹ J. A. Crisp,¹⁴ E. M. Stolper,¹⁵ D. J. Des Marais,¹ P. Sarrazin,³⁰ MSL Science Team‡

The Rocknest aeolian deposit is similar to aeolian features analyzed by the Mars Exploration Rovers (MERs) Spirit and Opportunity. The fraction of sand <150 micrometers in size contains ~55% crystalline material consistent with a basaltic heritage and ~45% x-ray amorphous material. The amorphous component of Rocknest is iron-rich and silicon-poor and is the host of the volatiles (water, oxygen, sulfur dioxide, carbon dioxide, and chlorine) detected by the Sample Analysis at Mars instrument and of the fine-grained nanophase oxide component first described from basaltic soils analyzed by MERs. The similarity between soils and aeolian materials analyzed at Gusev Crater, Meridiani Planum, and Gale Crater implies locally sourced, globally similar basaltic materials or globally and regionally sourced basaltic components deposited locally at all three locations.

The Mars Science Laboratory (MSL) rover Curiosity began exploring the surface of Mars on 6 August 2012 (universal time coordinated); until 13 September 2012, it conducted an initial engineering checkout of its mobility system, arm, and science instruments. Curiosity spent sols 57 to 100 (1) at a location named Rocknest, collecting and processing five scoops of loose, unconsolidated materials extracted from an aeolian sand shadow (2).

Five scoops of material from the Rocknest sand shadow were individually collected and sieved (<150 μm) by the Sample Acquisition, Sample Processing and Handling–Collection and Handling for In situ Martian Rock Analysis (SA/SPAH-CHIMRA) instrument (3). Scoops 1 and 2 were processed by CHIMRA and discarded to reduce (by entrainment and dilution) any terrestrial organic contamination that may have remained after a thorough cleaning on Earth (4) and to coat and passivate the interior surfaces of the collection device with Mars dust. Portions (40 to 50 mg) of scoops 3 and 4 were delivered to the Chemistry and Mineralogy (CheMin) instrument (5) and the “observation tray,” a 7.5-cm-diameter flat Ti-metal surface used for imaging and analyzing scooped and sieved material with Curiosity’s arm and mast instruments. Portions of scoop 5 were delivered to both CheMin and the Sample Analysis at Mars (SAM) quadrupole mass spectrometer/gas chromatograph/tunable laser spectrometer suite of instruments (6).

We describe the physical sedimentology of Rocknest and suggest possible sources for the

material making up the sand shadow. We use Alpha-Particle X-ray Spectrometer (APXS) and CheMin data to determine the amounts and chemistry of the crystalline and amorphous components of the sand shadow and compare these results with global soil measurements from the Mars Exploration Rovers (MERs) and to basaltic martian meteorites analyzed on Earth.

Results

Description and Interpretation of the Rocknest Sand Shadow

The Rocknest sand shadow (7) is an accumulation of wind-blown sediment deposited in the lower-velocity lee of an obstacle in the path of the wind. The orientation of the sand shadow indicates that the constructive winds were from the north. The surface is composed of dust-coated, predominantly rounded, very coarse (1- to 2-mm) sand grains (Fig. 1A). Trenches created during the scooping show that these larger grains form an armored surface ~2 to 3 mm in thickness (Fig. 1B). Beneath the armored surface, the bedform interior consists of finer-grained material whose size distribution extends through the resolution limit of Mars Hand Lens Imager (MAHLI) images (~30 μm per pixel under the conditions of the observation) (8). Because of CHIMRA’s 150- μm sieve, the larger grains that armor the surface could not be analyzed by CheMin.

Coarse sand grains that fell from the crust into the scoop-troughs lost their dust coating and show diversity in color, luster, and shape.

Among the grains are gray and red lithic fragments, clear/translucent crystal fragments, and spheroids with glassy luster (Fig. 1C). Some grains showed bright glints in the martian sunlight, suggesting specular reflections from mineral crystal faces or cleavage surfaces [similar features were observed by the optical microscope on board the Mars Phoenix Lander (9)]. MAHLI images of a sieved portion of material deposited on the observation tray (3) showed a variety of particle types from clear to colored to dark, angular to spherical, and dull to glassy-lustered (Fig. 1D).

During the scooping process, fragments of the armored surface were cohesive to the extent that “rafts” of surface crust were laterally compressed and displaced forward, and fragments of the crust fell into the scoop hole as cohesive units (Fig. 1B). The surface crust was also fractured and broken into rafts during scuffing by rover wheels (a process by which an excavation is made into the sub-surface of unconsolidated regolith by rotating a single rover wheel). Material beneath the crust also had some cohesion, as shown by the oversteep walls of the scoop scars (much greater than the angle of repose and vertical in some cases).

The sand shadow has a discernible internal structure. On the headwall and flanks of each scoop trench, a lighter-tone layer is apparent ~1 cm beneath and parallel to the dune surface (Fig. 1B). The origin of the layering is not understood, and three hypotheses are viable. First,

¹National Aeronautics and Space Administration (NASA) Ames Research Center, Moffett Field, CA 94035, USA. ²NASA Johnson Space Center, Houston, TX 77058, USA. ³Department of Geological Sciences, University of Texas, Austin, TX 78712, USA. ⁴Department of Geology, University of Arizona, Tucson, AZ 85721, USA. ⁵Department of Geological Sciences, Indiana University, Bloomington, IN 47405, USA. ⁶Malin Space Science Systems, San Diego, CA 92191, USA. ⁷U.S. Geological Survey, Santa Cruz, CA 95060, USA. ⁸Max-Planck-Institut für Sonnensystemforschung, 37191 Katlenburg-Lindau, Germany. ⁹Niels Bohr Institute, University of Copenhagen, 2100 Copenhagen, Denmark. ¹⁰Center for Radiophysics and Space Research, Cornell University, Ithaca, NY 14850, USA. ¹¹University of Guelph, Guelph, Ontario, N1G2W1, Canada. ¹²Lunar and Planetary Institute, Houston, TX 77058, USA. ¹³State University of New York–Stony Brook, Stony Brook, NY 11790, USA. ¹⁴Jet Propulsion Laboratory/California Institute of Technology, Pasadena, CA 91109, USA. ¹⁵California Institute of Technology, Pasadena, CA 91125, USA. ¹⁶Planetary Science Institute, Tucson, AZ 85719, USA. ¹⁷Chesapeake Energy, Oklahoma City, OK 73102, USA. ¹⁸University of California, Davis, CA 95616, USA. ¹⁹Institut de Recherche en Astrophysique et Planétologie (IRAP), UPS-OMP-CNRS, 31028 Toulouse, France. ²⁰Oregon State University, Corvallis, OR 97331, USA. ²¹Finnish Meteorological Institute, FI-00101 Helsinki, Finland. ²²NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA. ²³Rensselaer Polytechnic Institute, Troy, NY 12180, USA. ²⁴Geophysical Laboratory, Carnegie Institution of Washington, Washington, DC 20015, USA. ²⁵Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 México D.F. 04510, México. ²⁶Department of Earth and Planetary Sciences, University of Tennessee, Knoxville, TN 37996, USA. ²⁷The Johns Hopkins University Applied Physics Laboratory, Laurel, MD 20723, USA. ²⁸Princeton University, Princeton, NJ 08544, USA. ²⁹Arizona State University, Phoenix, AZ 85004, USA. ³⁰SETI Institute, Mountain View, CA 94043, USA.

*Corresponding author. E-mail: david.blake@nasa.gov

†Present address: Department of Earth and Planetary Sciences, University of California, Santa Cruz, CA 95064, USA.

‡MSL Science Team authors and affiliations are listed in the supplementary materials.

Curiosity at Gale Crater

the layering may represent changes in bulk composition or grain size that occurred during deposition. Second, the layering may be the result of changes in oxidation state or other chemical properties that occurred after deposition, in which case the conformable nature of the banding and the surface of the sand shadow reflect depth-dependent postdepositional chemical processes. Finally, the layering may represent zones richer or poorer in light-toned dust, reflecting times of lesser or greater sand accumulation relative to the air-fall dust.

The aeolian bedform at Rocknest is quite similar to coarse-grained ripples encountered at Gusev by the MER Spirit (10, 11) and at Meridiani Planum by the MER Opportunity (12, 13) in that a coarse-grained, indurated, dust-coated surface overlies an interior of markedly finer sediment. Coarse-grained ripples on Earth typically consist

of a surface veneer of coarse grains and a finer-grained interior (7, 14), and the martian bedforms have been considered analogous features (13, 15). The spatial grain-size sorting within coarse-grained ripples is thought to arise because of the short grain excursion length of the coarse grains traveling in creep and the much longer excursion length of finer saltating grains (16). With ripple migration, coarse grains are recycled through the bedform and become concentrated on the ripple surface, where impacts from saltating grains tend to buoy the grains upward.

Although the dynamics of sand shadows differ from those of coarse-grained ripples, and sand shadows on Earth do not characteristically show a coarse-grained surface, similar dynamics may arise owing to the mix-load transport of grains in creep and saltation. Alternate interpretations are also possible. First, the coarse-grained surface

could represent a lag formed as winds deflated finer grains. However, the paucity of coarse grains within the interior indicates that an unreasonable amount of deflation would have had to occur to produce the veneer. Second, the coarse-grained veneer could represent the terminal growth phase of the bedform. Because the size of a sand shadow is fixed by the upwind obstacle size (17), once the terminal size is approached, the lower wind speeds that characterize the wake and allow for deposition of finer sediment are replaced by wind speeds that approach the unmodified (primary) winds. At this point, there would be selective deposition of coarse grains traveling in creep, whereas finer saltating grains would bypass the bedform. Third, the sand shadow could have formed largely by the more readily transported fine saltation load, but as the area became depleted in finer grains, more of the residuum of

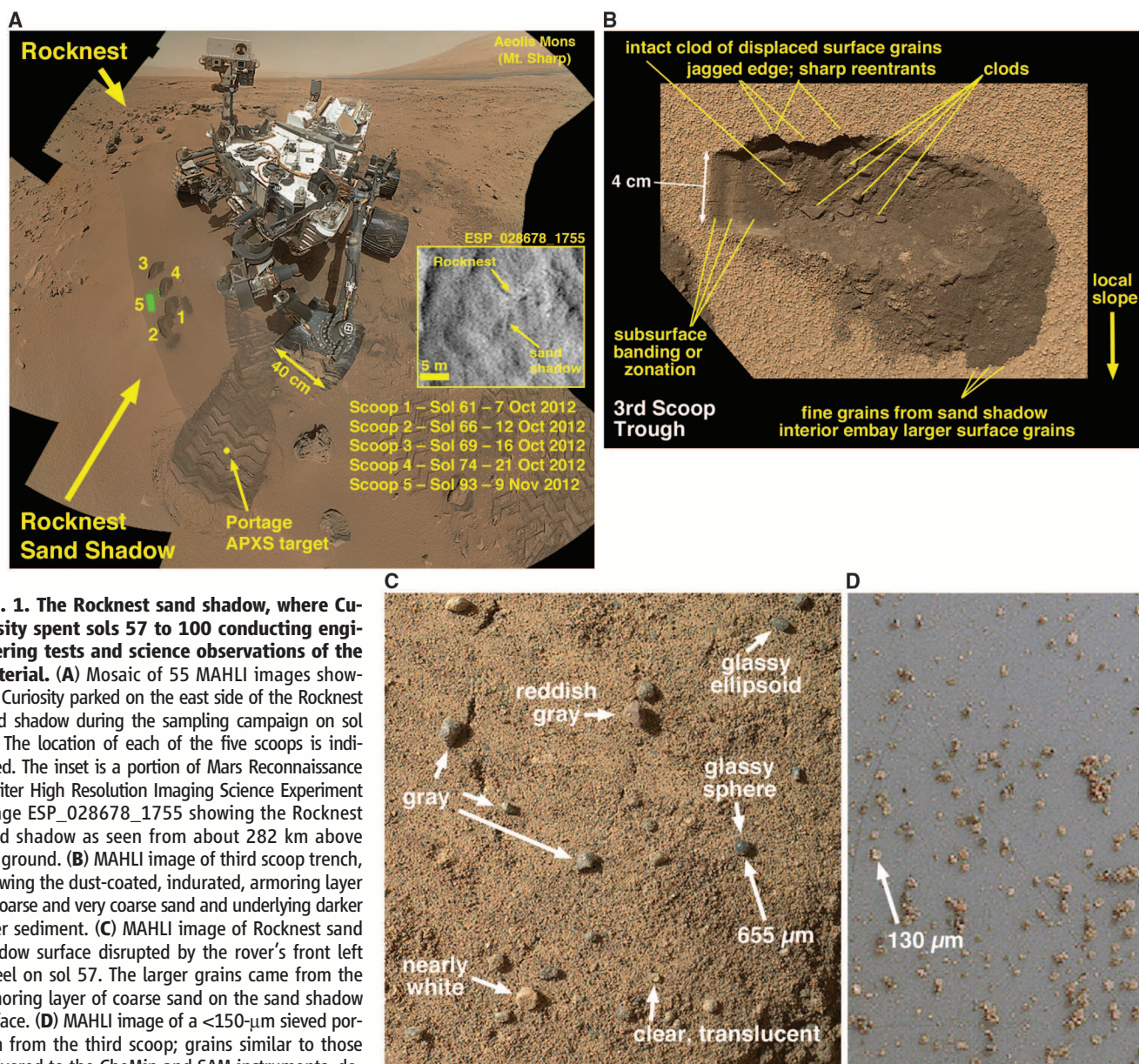


Fig. 1. The Rocknest sand shadow, where Curiosity spent sols 57 to 100 conducting engineering tests and science observations of the material. (A) Mosaic of 55 MAHLI images showing Curiosity parked on the east side of the Rocknest sand shadow during the sampling campaign on sol 84. The location of each of the five scoops is indicated. The inset is a portion of Mars Reconnaissance Orbiter High Resolution Imaging Science Experiment image ESP_028678_1755 showing the Rocknest sand shadow as seen from about 282 km above the ground. (B) MAHLI image of third scoop trench, showing the dust-coated, indurated, armoring layer of coarse and very coarse sand and underlying darker finer sediment. (C) MAHLI image of Rocknest sand shadow surface disrupted by the rover's front left wheel on sol 57. The larger grains came from the armoring layer of coarse sand on the sand shadow surface. (D) MAHLI image of a <150-μm sieved portion from the third scoop; grains similar to those delivered to the CheMin and SAM instruments, delivered to Curiosity's Ti observation tray.

coarser grains would be incorporated into transport, with the coarse-grained surface arising through subsequent deflation.

None of these interpretations explains the general absence of observed coarse grains in the interior; the contrast in grain size between the surface and the interior is more marked in the Rocknest sand shadow and in some of the coarse-grained ripples observed by MERs than in many Earth examples. This may reflect the greater impact energy of saltating grains on Mars compared with Earth and their ability to transport disproportionately larger grains in creep (18). Regarding the apparent absence of interior coarse grains, the small scooped areas may not be representative of the entire bedform, and interior horizons of coarse grains could easily have been bypassed. In addition, as seen with coarse-grained ripples on Earth, the amount of coarse sediment occurring in the interior varies and decreases with the supply of coarse grains.

Regardless of the origin of the coarse-grained surface, this armored surface would stabilize the bedform during all but the strongest wind events. In turn, the armored surface would allow time for surface induration to develop, further stabilizing the sand shadow. The similarity of the armoring and induration of the sand shadow at Rocknest to coarse-grained ripples encountered by Spirit and Opportunity suggests that the processes of grain transport and stabilization are similar across equatorial Mars and that Mars' winds (in recent eras) rarely were strong enough to transport sand grains of 1- to 3-mm diameter. To move the grains at the current atmospheric pressure of 0.02 kg/m³, the wind velocities would need to be ~36 m/s (80 mph) and ~52 m/s (116 mph), with and without saltation, respectively.

Under conditions of high obliquity, during which time the atmospheric pressure could increase to 0.04 kg/m³, these values would decrease to 26 m/s (58 mph) and ~37 m/s (83 mph), respectively (see Materials and Methods). The potential antiquity of the Rocknest sand shadow is highlighted by comparing it with granule ripples on Meridiani Planum, where cratering postdates a field of pristine granule ripples and the crater count suggests an age of 50,000 to 200,000 years (19).

Mineralogy of the Rocknest Sand Shadow

Analysis and interpretation of the mineralogy of the Rocknest sand shadow is given in Bish *et al.* (20). Rocknest consists of both crystalline and x-ray amorphous components. The crystalline component is basaltic, composed of plagioclase feldspar, forsteritic olivine, and the pyroxenes augite and pigeonite (20). All of the minor phases are consistent with a basaltic heritage, with the exception of anhydrite and hematite. By constraining the compositions of the individual crystalline phases on the basis of their measured unit-cell parameters, the chemical compositions of the minerals of Rocknest were determined (21, 22).

The crystalline component of Rocknest is chemically and mineralogically similar to that inferred for martian basalts across the planet and many of the basalts found in martian meteorites (Table 1) and, apart from somewhat lower Fe and K, broadly similar to estimates of the average martian crust (23). These basalts all contain (or have chemical compositions consistent with) the minerals olivine, augite, pigeonite, and plagioclase feldspar. The mineral proportions of the crystalline component of Rocknest are virtually identical to those calculated for the

unaltered Adirondack class basalts from Gusev Crater (CIPW normative mineralogy from their APXS analyses) (Table 1) (24, 25). Chemically, the mafic minerals of the Rocknest sediment (olivine, augite, and pigeonite) are all consistent with high-temperature chemical equilibria among Ca, Fe, and Mg at 1050 ± 75°C (Fig. 2). This consistency with chemical equilibria suggests, but does not prove, that these minerals and the plagioclase feldspar all derived from a common basaltic source rock, which was broken down into individual grains or lithic fragments and transported to Rocknest from regional source areas.

Bulk Chemistry of the Rocknest Sand Shadow

APXS provided an independent means of determining bulk chemistry of material in the Rocknest sand shadow. A measurement was made in a wheel scuff named Portage, which was largely devoid of surface crust (Fig. 1A). The chemical composition (taking into account analytical uncertainty) is within 2 SD of MER APXS analyses of basaltic soils (Table 2). The APXS chemistry of basaltic soils analyzed by the MERs at Gusev Crater and Meridiani Planum landing sites (Table 2) are within 1 SD of each other except for MgO and Na₂O, which are the same within 2 SD (24–28). The MER compositional averages exclude soils that contain a substantial local component (high SO₃ and high SiO₂ for Gusev and high Fe₂O₃ for Meridiani). The near identity of compositions of the Rocknest, Gusev, and Meridiani basaltic soils implies either global-scale mixing of basaltic material or similar regional-scale basaltic source material or some combination thereof.

Table 1. Mineralogy of Rocknest soil [CheMin x-ray diffraction (XRD)] and normative mineralogies of basaltic materials from Gusev Crater and of martian meteorites. (Rocknest data are amorphous-free values.) Rocknest soil by CheMin (20), average of scoop 5, proportions of crystalline phases normalized to 100%; values in italics uncertain. CIPW norms (weight) for Gusev basaltic materials from MER APXS chemical analyses (26), ignoring S and Cl; Fe³⁺/Fe_{tot} for Backstay and Irvine taken as 0.17, the value for an Adirondack basalt surface ground flat with the MER Rotary Abrasion Tool (RAT) (26). CIPW norms (wt %) of martian meteorites from bulk compositions; Fe³⁺/Fe_{tot} as

analyzed for Shergotty and Elephant Moraine (EETA) 79001A, estimated at 0.1 for Northwest Africa (NWA) 6234 and 0 for Queen Alexandra Range (QUE) 94201. K-spar is sanidine for the Rocknest soil, and normative orthoclase for others. Low-Ca Pyx is pigeonite for the soil and normative hypersthene for others. High-Ca Pyx is augite for the soil and normative diopside for others. Fe-Cr oxide includes magnetite, hematite, and chromite. All phosphorus in analyses are calculated as normative apatite. Mg no. is the % magnesium substituting for iron in the olivine structure, An refers to the % Ca substituting for Na in the plagioclase structure.

Location	Gale	Gusev			Meteorites			
Sample	Rocknest sand shadow	Adirondack	Backstay	Irvine	Shergotty	NWA 6234	EETA 79001A	QUE 94210
Quartz	1.4	0	0	0	0.2	0	0	3
Plagioclase	40.8	39	49	32	23	19	19	32
K-spar	1.3	1	6	6	1	0.5	0	0
Low-Ca Pyx	13.9	15	14	21	46	30	47	15
High-Ca Pyx	14.6	15	5	13	25	16	16	38
Olivine	22.4	20	15	16	0	27	13	0
Fe-Cr oxides	3.2	6	4	6	3	4	2	0
Ilmenite	0.9	1	2	2	2	2	1	4
Apatite	—	1	3	2	2	2	1	6
Anhydrite	1.5							
Mg no.	61 ± 3	57	62	55	51	63	63	40
An	57 ± 3	42	29	19	51	50	60	62

In contrast to the APXS measurement at the Portage wheel scuff, both CheMin and SAM measurements were carried out on the sieved, <150-μm-size fraction of soil. To discriminate potential differences between the fines delivered to CheMin and SAM and the bulk material analyzed in the wheel scuff, APXS chemistry was obtained from portions of sieved material deposited on the observation tray. APXS spectra from the bulk and sieved material are nearly identical, with the exception of a prominent Ti peak

and increased background from the observation tray (reflecting Ti metal of the tray). Additionally, Ca, Mn, and Fe signals in spectra from the observation tray are lowered proportionally as a function of their atomic number, which suggests that a fraction of these grains is smaller than the APXS sampling depth (29). Slightly elevated S and Cl, with a S/Cl ratio similar to that found in soils by MERs (30), suggest a potential enrichment of these two elements in the <150-μm fraction delivered to the observation tray.

To determine the amount and composition of the amorphous component, mass balance calculations were performed using the chemical composition of the bulk sample, the chemical compositions of the individual phases (e.g., plagioclase, sanidine, and olivine) and the relative proportions of those phases in the crystalline component. The empirical formulas of the major crystalline phases (Table 3) and their chemical compositions (table S2) were calculated from cell parameter data (20, 21) (table S1). The chemical formulas and compositions of the minor crystalline components were assigned by stoichiometry (e.g., ilmenite as TiFeO₃). The relative proportions of amorphous and crystalline components and their respective bulk compositions are summarized in Table 4, with Rocknest having ~45 weight percent (wt %) amorphous and ~55 wt % crystalline components (31). The chemical compositions and proportions of amorphous and crystalline components were calculated on a light-element-free basis. The relative proportion of the amorphous component will in reality be greater than 45 wt % because the volatile inventory is associated with that component (32).

Abundance estimates for the x-ray amorphous component of a sample may vary considerably, depending on the method used for their determination. Bish *et al.* (20), for example, used a full pattern-fitting method together with known amorphous standard materials analyzed in the laboratory to determine the amount of amorphous or poorly crystalline material contained in the CheMin x-ray diffraction pattern. Their reported value of ~27 wt % ± 50% (1 SD range of 13 to 40 wt %), as calculated from diffraction and scattering data alone, is somewhat lower than the ~45% calculated from mass balance considerations, but both values are within the combined analytical uncertainty of the two techniques.

The inferred chemical composition of the amorphous component (Table 4) contains ~23% FeO + Fe₂O₃, suggesting that ferric nanophase oxide [npOx (25, 26, 33)] is present in abundance. Similarly, S (principally contained within the amorphous component) is closely associated with the npOx in dunes at the MER sites (24, 27) as well. Abundances of SO₃ and Cl are correlated in soils from Gusev and Meridiani, which implies that both are associated with npOx in the amorphous component because these elements are not associated with Mg, Ca, or Fe in crystalline phases. The elements Cr, Mn, and P were associated with the amorphous component (Table 4), but

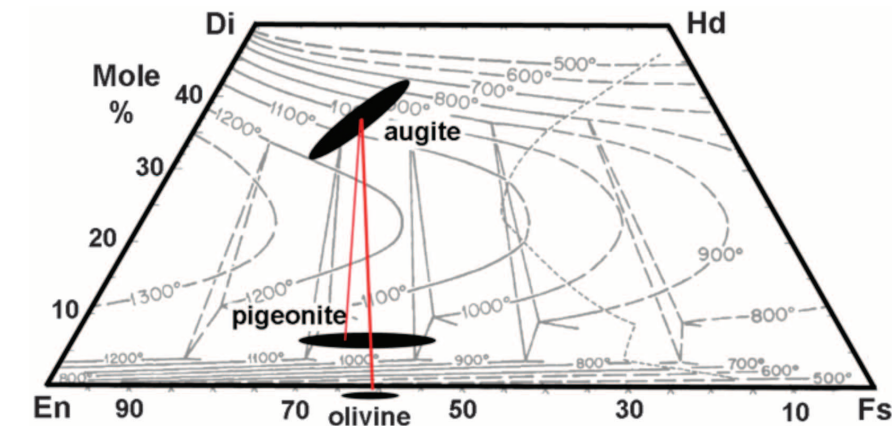


Fig. 2. Pyroxene compositional quadrilateral, showing the chemical and thermal relations between the major igneous minerals in the Rocknest sand shadow. Compositions of augite, pigeonite, and olivine in the Rocknest dune material, plotted on the pyroxene quadrilateral. En, enstatite, Mg₂Si₂O₆; Di, diopside, CaMgSi₂O₆; Hd, hedenbergite, CaFeSi₂O₆; and Fs, ferrosilite, Fe₂Si₂O₆. Pyroxenes are plotted within the quadrangle, based on CheMin XRD unit-cell parameters; olivine is plotted below the quadrilateral at the appropriate molar Mg/Fe ratio (20). Ellipses for each mineral approximate the uncertainties in mineral compositions from their unit-cell parameters. Gray background lines represent the surface of the pyroxene solvus, with temperatures in °C (40). Red lines are approximate equilibrium tie lines from the augite centroid composition to compositions of olivine and pigeonite, based on similar tie lines in an equilibrated anorthosite in lunar sample 62236 (41).

Table 2. Basaltic soil compositions from APXS analyses for Rocknest Portage, Gusev Crater, and Meridiani Planum.

	Rocknest	Gusev	Meridiani
Number	1*	48†	29†
SiO ₂ (wt %)	42.88 ± 0.47	46.1 ± 0.9	45.7 ± 1.3
TiO ₂	1.19 ± 0.03	0.88 ± 0.19	1.03 ± 0.12
Al ₂ O ₃	9.43 ± 0.14	10.19 ± 0.69	9.25 ± 0.50
Cr ₂ O ₃	0.49 ± 0.02	0.33 ± 0.07	0.41 ± 0.06
Fe ₂ O ₃ + FeO	19.19 ± 0.12	16.3 ± 1.1	18.8 ± 1.2
MnO	0.41 ± 0.01	0.32 ± 0.03	0.37 ± 0.02
MgO	8.69 ± 0.14	8.67 ± 0.60	7.38 ± 0.29
CaO	7.28 ± 0.07	6.30 ± 0.29	6.93 ± 0.32
Na ₂ O	2.72 ± 0.10	3.01 ± 0.30	2.21 ± 0.18
K ₂ O	0.49 ± 0.01	0.44 ± 0.07	0.48 ± 0.05
P ₂ O ₅	0.94 ± 0.03	0.91 ± 0.31	0.84 ± 0.06
SO ₃	5.45 ± 0.10	5.78 ± 1.25	5.83 ± 1.04
Cl	0.69 ± 0.02	0.70 ± 0.16	0.65 ± 0.09
Br (μg/g)	26 ± 6	53 ± 46	100 ± 111
Ni	446 ± 29	476 ± 142	457 ± 97
Zn	337 ± 17	270 ± 90	309 ± 87
Sum (wt %)	99.85	99.88	99.88
Cl/SO ₃	0.13 ± 0.02	0.12 ± 0.02	0.11 ± 0.01

*Gellert *et al.*, 2013 (35); analytical uncertainty. †±1SD of average.

Table 3. Empirical chemical formulas of the four major phases identified in the Rocknest soil estimated by crystal-chemical techniques.

Phase	Formula
Olivine	(Mg _{0.62(3)} Fe _{0.38}) ₂ SiO ₄
Plagioclase	(Ca _{0.57(13)} Na _{0.43})(Al _{1.57} Si _{2.43})O ₈
Augite	(Ca _{0.75(4)} Mg _{0.88(10)} Fe _{0.37})Si ₂ O ₆
Pigeonite	(Mg _{1.13(9)} Fe _{0.68(10)} Ca _{0.19})Si ₂ O ₆

they could instead be present as crystalline phases (e.g., Ca-phosphate and chromite) at abundances below the CheMin detection limit and/or as substitutional impurities in the major crystalline phases (e.g., Mn and Cr in pyroxene).

The SAM instrument analyzed Rocknest for volatile species and organic molecules (32), and it detected, in order of decreasing abundance, H₂O, SO₂, CO₂, and O₂. The crystalline phases, aside from a minor anhydrite component, do not include these species as a part of their structure, so they must either be present in the amorphous component or be present in the crystalline component at levels below the XRD detection limit, or both.

ChemCam spot observations in the scoop walls of Rocknest are characterized by the strong emissions from elemental hydrogen, although ChemCam is not sensitive to its bonding state (34). Comparison of this result with those of CheMin and SAM suggests that ChemCam detections of hydrogen most likely correspond to the H₂O associated with the amorphous component detected by CheMin.

Discussion

Global, Regional, and Local Sources

The crystalline phases in the Rocknest fines are consistent with a basaltic source and fit well within the measured qualitative mineralogy of basaltic martian meteorites and the normative mineralogy of Adirondack class olivine basalts at Gusev Crater (25) (Table 1). If the Rocknest

assemblage of basaltic crystalline and amorphous components is locally derived, it is distinct from mafic float rocks analyzed to date by APXS and ChemCam in Gale Crater (34, 35). This observation suggests that the similarity in the chemical compositions of aeolian bedforms (basaltic soil) at Gale, Gusev, and Meridiani (Table 2) might result from global-scale aeolian mixing of local-to-regional basaltic material that may or may not have variable chemical compositions. This process would require sufficiently strong winds occurring with sufficient frequency over a long enough time to achieve global or regional-scale transport of grains by saltation and suspension.

An alternative explanation for the comparable chemical compositions of aeolian bedforms at Gale, Gusev, and Meridiani is that the chemical compositions of martian basalts are similar at regional scales everywhere on the planet. The Rocknest sand shadow could reasonably have locally sourced 1- to 2-mm particles, with finer-grained regional basaltic material plus a contribution from global dust. The similarity of soil compositions (Table 2) suggests that the basaltic fine-grained materials at Gusev, Meridiani, and Gale Crater provide a reasonable approximation to the bulk composition of the exposed martian crust (36, 37).

It is tempting to suggest that the light-toned martian dust is largely represented by the Rocknest amorphous component. However, we have no data to show that the <150-μm size fraction (clay to fine-sand size fraction) of material analyzed

by CheMin has its finest material preferentially enriched in amorphous material. The evidence from MER for basaltic soils suggests that the chemical composition of the fine-grained, light-toned soil is approximately the same as the coarser-grained, dark-toned soils [e.g., table 10 in (38)].

The central mound of Gale Crater (Mt. Sharp or Aeolis Mons) exhibits reflectance spectra suggesting the presence of crystalline hydrated sulfate minerals and phyllosilicates (39), but neither was seen in Rocknest (above the 1 to 2% level). The absence of material from Mt. Sharp could arise from the wind pattern during formation of the Rocknest sand shadow; it is oriented so as to imply sediment transport from the north, and Mt. Sharp is east and southeast of Rocknest.

Materials and Methods

Calculation of Wind Speeds Required to Form the Rocknest Sand Shadow

The wind velocity required to move the coarse grains of the sand shadow by creep can be calculated. The critical shear velocity (u_{*c}) of the wind needed to transport 1-mm-diameter (d) grains is given by (42) as

$$u_{*c} = \sqrt{0.0123 \left(sgd + \frac{0.0003 \text{ kg/s}^2}{\tilde{n}_f d} \right)}$$

where $s = \tilde{n}_s/\tilde{n}_f$, $\tilde{n}_s$ is the density of the grains using basalt (3000 kg/m³), $\tilde{n}_f$ is the density of

Table 4. Chemical composition and proportion of XRD amorphous component in Rocknest Portage from APXS and CheMin data.

	Origin		Remove XRD crystalline component*										Composition	
	APXS†	APXS+CheMin	Plagioclase	Sanidine	Olivine	Augite	Pigeonite	Ilmenite	Hematite	Magnetite	Anhydrite	Quartz	Amorphous‡	Crystalline
SiO ₂ , wt %	42.88	42.88	30.88	30.42	25.95	21.63	17.51	17.51	17.51	17.51	17.51	16.76	37.20	47.59
TiO ₂	1.19	1.19	1.19	1.19	1.19	1.19	1.19	0.93	0.93	0.93	0.93	0.93	2.06	0.47
Al ₂ O ₃	9.43	9.43	2.85	2.72	2.72	2.72	2.72	2.72	2.72	2.72	2.72	2.72	6.04	12.24
Cr ₂ O ₃	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	1.09	0.00
FeO+Fe ₂ O ₃ §	19.19	10.43	10.43	10.43	10.43	10.43	10.43	10.43	10.43	10.43	10.43	10.43	23.14	-0.10
FeO-Cryst	—	7.37	7.37	7.37	3.31	2.29	0.59	0.35	0.35	0.00	0.00	0.00	-0.01	13.48
Fe ₂ O ₃ -Cryst	—	1.39	1.39	1.39	1.39	1.39	1.39	1.39	0.79	0.00	0.00	0.00	-0.01	2.55
MnO	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.91	0.00
MgO	8.69	8.69	8.69	8.69	4.97	3.72	2.19	2.19	2.19	2.19	2.19	2.19	4.86	11.86
CaO	7.28	7.28	4.65	4.65	4.65	3.19	2.87	2.87	2.87	2.87	2.53	2.53	5.61	8.67
Na ₂ O	2.72	2.72	1.62	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60	3.56	2.03
K ₂ O	0.49	0.49	0.49	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.89	0.16
P ₂ O ₅	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	2.09	-0.01
SO ₃	5.45	4.96	4.96	4.96	4.96	4.96	4.96	4.96	4.96	4.96	4.96	4.96	11.01	-0.05
SO ₃ -Cryst [#]	—	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.00	0.00	-0.01	0.90
Cl	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	1.35	-0.01
Sum	99.77	99.77	77.47	76.77	64.52	56.47	48.80	48.30	47.70	46.55	45.71	44.96	99.77	99.77
Σ(FeO+Fe ₂ O ₃)	19.19	19.19	—	—	—	—	—	—	—	—	—	—	23.14	16.03
Σ(SO ₃)	5.54	5.54	—	—	—	—	—	—	—	—	—	—	11.01	0.90
Relative to whole sample			22.3	0.7	12.3	8.0	7.6	0.5	0.6	1.2	0.8	0.8	45.3	54.7
Relative to XRD crystalline			40.8	1.3	22.4	14.6	13.9	0.9	1.1	2.1	1.5	1.4	—	100.0

*Plagioclase, An57; Olivine, Fo62; Augite, En44Fs20Wo36 (Mg/Fe, 2.2 atomic); Pigeonite, En56Fs35Wo8 (Fe/Mg, 1.6 atomic).

†APXS chemistry from Gellert *et al.* (35).

‡Cr₂O₃ and MnO calculated with the amorphous component.

§Total Fe as FeO+Fe₂O₃ because APXS does not distinguish oxidation states.

||FeO required for Fe²⁺ crystalline phases (olivine, augite, pigeonite, ilmenite, and magnetite).

||Fe₂O₃ required for Fe³⁺ crystalline phases (hematite and magnetite).

#SO₃ required for crystalline SO₃ crystalline phase (anhydrite).

martian air (0.02 kg/m^3), and g is the acceleration due to gravity (3.71 m/s^2). The calculated u_{*c} is 2.6 m/s , which represents the fluid shear velocity to initiate motion. Because grains in creep derive a portion of their momentum from collisions by saltating grains, on Earth once saltation begins, creep can occur down to $0.7 u_{*c}$ (1.8 m/s as applied to the Rocknest grains), which represents the impact threshold for motion. Given a boundary layer created by winds blowing over the surface, shear velocities can then be related to the wind speeds above the surface by the law of the wall

$$u_z = \frac{u_*}{k} \ln\left(\frac{z}{z_0}\right)$$

where u_z is the wind speed at height z above the surface (taken here as 1 m), k is a constant of 0.407 , and z_0 is the roughness height where the idealized logarithmic wind profile is predicted to be zero. Roughness height varies by grain size and the height of surface features, such as wind ripples (7), and also by the height and intensity of the saltation cloud (43). Rocknest conditions are unknown, but z_0 is taken as 0.3 mm , which would be the roughness height with wind ripples 10 mm in height. Estimated wind speeds at 1 m above the surface are $\sim 52 \text{ m/s}$ (116 mph) and 36 m/s (80 mph), without and with saltation, respectively. As a result of the lower gravity and reduced atmospheric density on Mars, a greater hysteresis exists than on Earth between the fluid and impact thresholds, and saltation impacts upon grains are more energetic (18, 44, 45). The combined effects suggest that initial transport of the coarse surface grains probably occurred at lower wind speeds than those calculated. Conversely, reactivation of the sand shadow would require considerably higher wind speeds because of induration of the surface.

Although observations from the Viking Lander 1 suggest that wind speeds of 30 m/s at a height of 1.6 m occurred during its 2-year lifetime (46), we do not know how often Mars winds can be capable of transporting $1\text{--}2\text{-mm}$ grains. The wind estimates above suggest that formation of the Rocknest sand shadow has involved rare strong winds and that reactivation of the sand shadow from its currently indurated state would require even stronger and rarer winds.

Given the possibility of considerable antiquity of the Rocknest sand shadow and similar coarse-grained bedforms on Mars, could their activation correspond to the martian obliquity cycle? At low obliquities, the atmosphere collapses onto the polar caps, but at high obliquity, CO_2 is released to the atmosphere (47, 48). Taken as an end member, atmospheric density may double at high obliquity and thereby enhance aeolian activity (48). As a comparison with the above values calculated for the present martian atmosphere, using 0.04 kg/m^3 for atmospheric density, the calculated fluid u_{*c} is 1.9 m/s and the impact u_{*c} is 1.3 m/s , which correspond to wind speeds at

the 1-m height of $\sim 37 \text{ m/s}$ (83 mph) and 26 m/s (58 mph), respectively. Although considerably lower than values calculated for present conditions, rare strong wind events are still implied.

References and Notes

1. A Mars solar day has a mean period of 24 hours, 39 min, 35 s and is customarily referred to as a "sol" to distinguish it from the roughly 3% shorter day on Earth.
2. A sand shadow is an accumulation of wind-blown sediment deposited in the lower-velocity lee of an obstacle in the path of the wind.
3. R. C. Anderson *et al.*, Collecting samples in Gale Crater, Mars; An overview of the Mars Science Laboratory Sample Acquisition, Sample Processing and Handling System. *Space Sci. Rev.* **170**, 57–75 (2012). doi: [10.1007/s11214-012-9898-9](#)
4. M. S. Anderson *et al.*, In situ cleaning of instruments for the sensitive detection of organics on Mars. *Rev. Sci. Instrum.* **83**, 105109 (2012). doi: [10.1063/1.4757861](#); pmid: [23126806](#)
5. D. F. Blake *et al.*, Characterization and calibration of the CheMin mineralogical instrument on Mars Science Laboratory. *Space Sci. Rev.* **170**, 341–399 (2012). doi: [10.1007/s11214-012-9905-1](#)
6. P. R. Mahaffy *et al.*, The sample analysis at Mars investigation and instrument suite. *Space Sci. Rev.* **170**, 401–478 (2012). doi: [10.1007/s11214-012-9879-z](#)
7. R. A. Bagnold, *The Physics of Blown Sand and Desert Dunes* (Chapman and Hall, London, 1941).
8. K. S. Edgett *et al.*, Curiosity's Mars Hand Lens Imager (MAHLI) Investigation. *Space Sci. Rev.* **170**, 259–317 (2012). doi: [10.1007/s11214-012-9910-4](#)
9. W. Goetz *et al.*, Microscopic analysis of soils at the Phoenix landing site, Mars: Classification of soil particles and description of their optical and magnetic properties. *J. Geophys. Res.* **115**, E00E22 (2010). doi: [10.1029/2009JE003437](#)
10. K. E. Herkenhoff *et al.*, In situ observations of the physical properties of the martian surface, in *The Martian Surface: Composition, Mineralogy, and Physical Properties*, J. F. Bell III, Ed. (Cambridge Univ. Press, Cambridge, 2008), pp. 451–467.
11. R. Sullivan *et al.*, Wind-driven particle mobility on Mars: Insights from Mars Exploration Rover observations at "El Dorado" and surroundings at Gusev Crater. *J. Geophys. Res.* **113**, E06S07 (2008). doi: [10.1029/2008JE003101](#)
12. L. A. Soderblom *et al.*, Soils of Eagle Crater and Meridiani Planum at the Opportunity rover landing site. *Science* **306**, 1723–1726 (2004). doi: [10.1126/science.1105127](#); pmid: [15576606](#)
13. R. Sullivan *et al.*, Aeolian processes at the Mars exploration rover Meridiani Planum landing site. *Nature* **436**, 58–61 (2005). doi: [10.1038/nature03641](#); pmid: [16001061](#)
14. S. G. Fryberger, P. Hesp, K. Hastings, Aeolian granule ripple deposits, Namibia. *Sedimentology* **39**, 319–331 (1992). doi: [10.1111/j.1365-3091.1992.tb01041.x](#)
15. D. J. Jerolmack, D. Mohrig, J. P. Grotzinger, D. A. Fike, W. A. Watters, Spatial grain size sorting in eolian ripples and estimation of wind conditions on planetary surfaces: Application to Meridiani Planum, Mars. *J. Geophys. Res.* **111**, E12S02 (2006). doi: [10.1029/2005JE002544](#)
16. J. M. Ellwood, P. D. Evans, I. G. Wilson, Small scale aeolian bedforms. *J. Sed. Petrol.* **45**, 554–561 (1975).
17. P. A. Hesp, The formation of shadow dunes. *J. Sed. Petrol.* **51**, 101–112 (1981).
18. M. P. Almeida, E. J. R. Parteli, J. S. Andrade Jr., H. J. Herrmann, Giant saltation on Mars. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 6222–6226 (2008). doi: [10.1073/pnas.0800202105](#); pmid: [18443302](#)
19. M. P. Golombek *et al.*, Constraints on ripple migration at Meridiani Planum from Opportunity and HiRISE observations of fresh craters. *J. Geophys. Res.* **115**, E00F08 (2010). doi: [10.1029/2010JE003628](#)
20. D. L. Bish *et al.*, X-Ray diffraction results from Mars Science Laboratory: Mineralogy of Rocknest at Gale Crater. *Science* **341**, 1238932 (2013); doi: [10.1126/science.1238932](#)
21. Supplementary materials are available on Science Online.
22. Unit cell parameters obtained from the RRUFF Project database, <http://rruff.info/ima>.
23. S. R. Taylor, S. M. McLennan, *Planetary Crusts: Their Composition, Origin and Evolution* (Cambridge Univ. Press, Cambridge, (2009).
24. R. V. Morris *et al.*, Iron mineralogy and aqueous alteration from Husband Hill through Home Plate at Gusev Crater, Mars: Results from the Mössbauer instrument on the Spirit Mars Exploration Rover. *J. Geophys. Res.* **113**, E12S42 (2008). doi: [10.1029/2008JE003201](#)
25. D. W. Ming *et al.*, Geochemical properties of rocks and soils in Gusev Crater, Mars: Results of the Alpha Particle X-ray Spectrometer from Cumberland Ridge to Home Plate. *J. Geophys. Res.* **113**, E12S39 (2008). doi: [10.1029/2008JE003195](#)
26. R. V. Morris *et al.*, Mössbauer mineralogy of rock, soil, and dust at Gusev Crater, Mars: Spirit's journey through weakly altered olivine basalt on the Plains and pervasively altered basalt in the Columbia Hills. *J. Geophys. Res.* **111**, E02S13 (2006). doi: [10.1029/2005JE002584](#)
27. A. S. Yen *et al.*, An integrated view of the chemistry and mineralogy of martian soils. *Nature* **436**, 49–54 (2005). doi: [10.1038/nature03637](#); pmid: [16001059](#)
28. A. S. Yen *et al.*, Evidence for a global martian soil composition extends to Gale Crater. *45th Lunar and Planetary Science Conference*, March 2013, Published on CD by the Lunar and Planetary Institute, Houston, Texas, Abstract 2495 (2013).
29. J. A. Berger *et al.*, MSL Titanium Observation Tray Measurements with APXS. *45th Lunar and Planetary Science Conference*, March 2013, Published on CD by the Lunar and Planetary Institute, Houston, Texas, Abstract 1321 (2013).
30. R. Gellert *et al.*, Alpha Particle X-ray Spectrometer (APXS): Results from Gusev Crater and calibration report. *J. Geophys. Res.* **111**, E02S05 (2006). doi: [10.1029/2005JE002555](#)
31. Because APXS does not discriminate among iron oxidation states, the total Fe concentration was proportioned in accordance with the oxidation state information carried by the crystalline phases (Table 3, column 3). FeO-Cryst and Fe₂O₃-Cryst are the concentrations of FeO and Fe₂O₃ required to accommodate olivine, augite, pigeonite, ilmenite, and magnetite and hematite, in accordance with their valence states. The remaining iron (FeO + Fe₂O₃) is then associated with the amorphous component without implications for oxidation state. Similarly, some SO₃ is reported as SO₃-Cryst to accommodate anhydrite as a crystalline component.
32. L. A. Leshin *et al.*, Volatile, isotope, and organic analysis of martian fines with the Mars Curiosity Rover. *Science* **341**, 1238937 (2013); doi: [10.1126/science.1238937](#)
33. Nanophase ferric oxide (npOx) is a generic name for amorphous, poorly crystalline, or short-range ordered products of oxidative alteration/weathering that have octahedrally coordinated Fe³⁺ (Mössbauer doublet) and are predominantly oxide/oxyhydroxide/hydrous in nature. Depending on local conditions, npOx (as encountered on Earth) can be any combination of superparamagnetic hematite and goethite, lepidocrocite, ferrihydrite, schwertmannite, akaganeite, hisingerite, and the octahedral Fe³⁺-rich particles that pigment iddingsite and palagonite. npOx can also incorporate anions like (SO₄)²⁻, Cl⁻, and (PO₄)³⁻ through specific chemical adsorption. Because of different local conditions on Mars, one or more forms of npOx on the planet may be uncommon or not present on Earth.
34. P.-Y. Meslin *et al.*, Soil diversity and hydration as observed by ChemCam at Gale Crater, Mars. *Science* **341**, 1238670 (2013); doi: [10.1126/science.1238670](#)
35. R. Gellert *et al.*, Initial MSL APXS activities and observations at Gale Crater, Mars, *45th Lunar and Planetary Science Conference*, March 2013, Published on CD by the Lunar and Planetary Institute, Houston, Texas, Abstract 1432 (2013).

36. H. Y. McSween Jr., G. J. Taylor, M. B. Wyatt, Elemental composition of the martian crust. *Science* **324**, 736–739 (2009). doi: [10.1126/science.1165871](https://doi.org/10.1126/science.1165871); pmid: [19423810](https://pubmed.ncbi.nlm.nih.gov/19423810/)
37. S. R. Taylor, S. M. McLennan, *Planetary Crusts: Their Composition, Origin and Evolution* (Cambridge Univ. Press, Cambridge, 2009).
38. R. V. Morris *et al.*, Mössbauer mineralogy of rock, soil, and dust at Meridiani Planum, Mars: Opportunity's journey across sulgate-rich outcrop, basaltic sand and dust, and hematite lag deposits. *J. Geophys. Res.* **111**, E12515 (2006). doi: [10.1029/2005JE002584](https://doi.org/10.1029/2005JE002584)
39. R. E. Milliken, J. P. Grotzinger, B. J. Thomson, Paleoclimate of Mars as captured by the stratigraphic record in Gale Crater. *GRL* **37**, L04201 (2010). doi: [10.1029/2009GL041870](https://doi.org/10.1029/2009GL041870)
40. D. H. Lindsley, Pyroxene thermometry. *Am. Mineral.* **68**, 477–493 (1983).
41. P. H. Warren, J. T. Wasson, The compositional-petrographic search for pristine nonmare rocks: Third foray. *Proc. Lunar Planet. Sci. Conf. 10th* (1979), 583–610.
42. Y. Shao, H. Lu, A simple expression for wind erosion threshold friction velocity. *J. Geophys. Res.* **105**, (D17), 22,437–22,443 (2000). doi: [10.1029/2000JD900304](https://doi.org/10.1029/2000JD900304)
43. P. R. Owen, Saltation of uniform grains in air. *J. Fluid Mech.* **20**, 225–242 (1964). doi: [10.1017/S0022112064001173](https://doi.org/10.1017/S0022112064001173)
44. P. Claudin, B. Andreotti, A scaling law for aeolian dunes on Mars, Venus, Earth, and for subaqueous ripples. *Earth Planet. Sci. Lett.* **252**, 30–44 (2006). doi: [10.1016/j.epsl.2006.09.004](https://doi.org/10.1016/j.epsl.2006.09.004)
45. J. F. Kok, Difference in the wind speeds required for initiation versus continuation of sand transport on Mars: Implications for dunes and dust storms. *Phys. Rev. Lett.* **104**, 074502 (2010). doi: [10.1103/PhysRevLett.104.074502](https://doi.org/10.1103/PhysRevLett.104.074502); pmid: [20366891](https://pubmed.ncbi.nlm.nih.gov/20366891/)
46. R. E. Arvidson, E. A. Guinness, H. J. Moore, J. Tillman, S. D. Wall, Three Mars years: Viking Lander 1 imaging observations. *Science* **222**, 463–468 (1983). doi: [10.1126/science.222.4623.463](https://doi.org/10.1126/science.222.4623.463); pmid: [17746178](https://pubmed.ncbi.nlm.nih.gov/17746178/)
47. C. E. Newman, S. R. Lewis, P. L. Read, The atmospheric circulation and dust activity in different orbital epochs on Mars. *Icarus* **174**, 135–160 (2005). doi: [10.1016/j.icarus.2004.10.023](https://doi.org/10.1016/j.icarus.2004.10.023)
48. R. J. Phillips *et al.*, Massive CO₂ ice deposits sequestered in the south polar layered deposits of Mars. *Science* **332**, 838–841 (2011). doi: [10.1126/science.1203091](https://doi.org/10.1126/science.1203091); pmid: [21512003](https://pubmed.ncbi.nlm.nih.gov/21512003/)

Acknowledgments: Support from the NASA Mars Science Laboratory Mission is gratefully acknowledged. The chemical and mineralogical data presented here are derived from the archived data sets in the NASA Planetary Data System (PDS) <http://pds-geosciences.wustl.edu/missions/msl>, specifically MSL-M-CHEMIN-2-EDR-V1.0 and MSL-M-APXS-2-EDR-V1.0. M.B.M. was funded by the Danish Council for Independent Research/Natural Sciences (Det Frie Forskningsråd Natur og Univers FNU grants 12-127126 and 11-107019). W.G. acknowledges partial funding by the Deutsche Forschungsgemeinschaft (DFG grant GO 2288/1-1). Some of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA.

Supplementary Materials

www.sciencemag.org/content/341/6153/1239505/suppl/DC1

Supplementary Text

Figs. S1 to S4

Tables S1 and S2

References

23 April 2013; accepted 31 July 2013

10.1126/science.1239505

Volatile, Isotope, and Organic Analysis of Martian Fines with the Mars Curiosity Rover

L. A. Leshin,^{1*} P. R. Mahaffy,² C. R. Webster,³ M. Cabane,⁴ P. Coll,⁵ P. G. Conrad,² P. D. Archer Jr.,⁶ S. K. Atreya,⁷ A. E. Brunner,^{2,8} A. Buch,⁹ J. L. Eigenbrode,² G. J. Flesch,³ H. B. Franz,^{2,10} C. Freissinet,² D. P. Glavin,² A. C. McAdam,² K. E. Miller,¹¹ D. W. Ming,⁶ R. V. Morris,⁶ R. Navarro-González,¹² P. B. Niles,⁶ T. Owen,¹³ R. O. Pepin,¹⁴ S. Squyres,¹⁵ A. Steele,¹⁶ J. C. Stern,² R. E. Summons,¹¹ D. Y. Sumner,¹⁷ B. Sutter,^{6,18} C. Szopa,⁴ S. Teinturier,⁴ M. G. Trainer,² J. J. Wray,¹⁹ J. P. Grotzinger,²⁰ MSL Science Team†

Samples from the Rocknest aeolian deposit were heated to ~835°C under helium flow and evolved gases analyzed by Curiosity's Sample Analysis at Mars instrument suite. H₂O, SO₂, CO₂, and O₂ were the major gases released. Water abundance (1.5 to 3 weight percent) and release temperature suggest that H₂O is bound within an amorphous component of the sample. Decomposition of fine-grained Fe or Mg carbonate is the likely source of much of the evolved CO₂. Evolved O₂ is coincident with the release of Cl, suggesting that oxygen is produced from thermal decomposition of an oxychloride compound. Elevated δ D values are consistent with recent atmospheric exchange. Carbon isotopes indicate multiple carbon sources in the fines. Several simple organic compounds were detected, but they are not definitively martian in origin.

The exchange of materials between a planet's interior, surface, and atmosphere drives the composition of mineral and chemical constituents that can create habitable environments on the terrestrial planets. Surface deposits, including

aeolian fines, form an important record of these material exchanges. Martian surface fines are especially interesting because previous chemical studies by the Viking landers, Pathfinder, Spirit, and Opportunity (1–4) show that the bulk chemical composition of these materials is relatively constant at widely spaced locations across the planet. This can result from a combination of mechanical mixing on global scales and a similarity in the chemical composition of bedrock and sediments on regional to global scales (5). The finer-grained fractions, in particular, may

provide information about the average composition of the martian crust (6).

The Sample Analysis at Mars (SAM) instrument suite onboard the Mars Science Laboratory (MSL) rover Curiosity provides diverse analytical capabilities for exploring martian materials, including volatile and isotopic compositions, and a search for organic compounds, whether of abiotic or biological origin (7). Traces of organic compounds have been found in martian meteorites (8–12), but previous landed missions, most notably Viking, did not find definitive evidence of martian organic material (13).

Curiosity's first sampling campaign took place at Rocknest, an aeolian sand shadow. The rover ingested fine-grained Rocknest material into its two analytical instruments: Chemistry and Mineralogy (CheMin), for x-ray diffraction, and SAM, for analysis of volatiles. Both SAM and CheMin sampled portions from scooped materials that were sieved to contain grain sizes <150 μ m. Mineralogical and chemical results summarized in a companion paper (14) indicate bulk composition similar to martian fines analyzed by previous missions. Plagioclase, olivine, augite, pigeonite, and minor magnetite are the major igneous minerals (15). Minor anhydrite and hematite are the only nonigneous minerals detected. Along with these crystalline phases, the chemical and mineralogical analyses indicate that almost half of the <150- μ m fraction comprises amorphous material (14). SAM performs evolved gas analysis (EGA) with the quadrupole mass spectrometer (QMS) and isotope measurements of evolved gases using both the QMS and the tunable laser spectrometer (TLS), the latter being sensitive to isotopes of CO₂ and H₂O. Organic analyses can be performed with the QMS alone or when it is coupled to the gas chromatograph (GC). SAM analyzed four separate portions from the fifth scooped sample at Rocknest

¹Department of Earth and Environmental Sciences and School of Science, Rensselaer Polytechnic Institute, Troy, NY 12180, USA. ²Planetary Environments Laboratory, NASA Goddard Space Flight Center, Greenbelt MD 20771, USA. ³Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA. ⁴LATMOS, UPMC Univ. Paris 06, Université Versailles St-Quentin, UMR CNRS 8970, 75005 Paris, France. ⁵LISA, Univ. Paris-Est Créteil, Univ. Paris Diderot and CNRS, 94000 Créteil, France. ⁶Astromaterials Research and Exploration Science Directorate, NASA Johnson Space Center, Houston, TX 77058, USA. ⁷Department of Atmospheric, Oceanic and Space Sciences, University of Michigan, Ann Arbor, MI 48109–2143, USA. ⁸Department of Astronomy, University of Maryland, College Park, MD 20742, USA. ⁹Laboratoire Génie des Procédés et Matériaux, Ecole Centrale Paris, 92295 Châtenay-Malabry, France. ¹⁰Center for Research and Exploration in Space Science and Technology, University of Maryland Baltimore County, Baltimore, MD 21250, USA. ¹¹Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. ¹²Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F. 04510, México. ¹³Institute for Astronomy, University of Hawaii, Honolulu, HI 96822, USA. ¹⁴School of Physics and Astronomy, University of Minnesota, Minneapolis, MN 55455, USA. ¹⁵Department of Astronomy, Cornell University, Ithaca, NY 14853, USA. ¹⁶Geophysical Laboratory, Carnegie Institution of Washington, Washington, DC 20015, USA. ¹⁷Department of Geology, University of California, Davis, CA 95616, USA. ¹⁸Jacobs, Houston, TX 77058, USA. ¹⁹School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA. ²⁰Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA.

*Corresponding author. E-mail: leshin@rpi.edu

†MSL Science Team authors and affiliations are listed in the supplementary materials.

Table 1. Experiment parameters for four analyses of Rocknest fines. All evolved gases were analyzed by the QMS; temperature (T) range of gases that were then sent to the GC and TLS are shown.

Rocknest run	Sol (mission day)	Sample T range of gas sent to GC (°C)	Sample T range of gas sent to TLS (°C)	Rationale
Run 1	93	146–533	547–702*	GC: Low-T organics TLS: Predicted T for thermal decomposition of carbonates
Run 2	96	98–425	440–601	GC: Low-T organics below SO ₂ evolution T TLS: Target CO ₂ from suspected carbonate peak
Run 3	99	533–822	234–425	GC: High-T organics TLS: Low-T CO ₂ and H ₂ O evolution
Run 4	117	251–289	350–443	GC: Narrow T cut for organics below O ₂ evolution T TLS: Narrow T cut targeting suspected carbonate

*Due to the low volume of gas released by Rocknest in this temperature range, isotope data were not obtained for this run.

(see Table 1 and Materials and Methods). The exact mass of each Rocknest portion delivered to SAM is not measured by Curiosity, but tests on Earth are consistent with 50 ± 8 mg per portion (16).

Results and Discussion

Volatile Release

The volatile compounds observed in EGA typically reflect a combination of processes including desorption of trapped volatiles, mineral thermal decomposition, and chemical reaction during heating of the samples (17, 18). Pure minerals and chemicals produce volatile products at predictable temperatures; however, in natural mixtures, these temperatures can be strongly shifted by physical characteristics of the samples (e.g., grain size) and by interactions between mineral and chemical components (17).

All four Rocknest analyses yielded H_2O , SO_2 , CO_2 , and O_2 , in descending order of average abundance (Fig. 1 and Table 2). H_2O , CO_2 , and O_2 abundances are relatively consistent from run to run and track each other within experimental uncertainty, whereas SO_2 abundance is variable from run to run. Repeated observation of H_2O , CO_2 , and O_2 gas abundances with similar values suggests that differences in sample mass cannot explain the heterogeneity in SO_2 abundance, and thus the variability must be due to variation in the abundance of S-bearing minerals in different portions.

The H_2O observed in Rocknest EGA comprises a broad peak centered at $\sim 300^\circ\text{C}$. Abundance estimates are ~ 1.5 to 3 weight percent (wt %) H_2O in the $<150\text{-}\mu\text{m}$ fraction. The peak temperature and breadth of the H_2O release is most consistent with bound H_2O in amorphous phases. Specifically, adsorbed H_2O , H_2O bound to amorphous phases (e.g., amorphous aluminosilicate materials, nanophase ferric oxides and oxyhydroxides), interlayer H_2O from phyllosilicates, dehydration of several salts, and dehydration of ferric oxyhydroxides could have contributed to the lower-temperature H_2O release (Fig. 2). Higher-temperature H_2O could result from more tightly bound structural H_2O and/or OH in minor minerals present below the CheMin detection limit, as well as H_2O occluded in minerals and glasses. However, if the water detected was released from a single host mineral, CheMin should have detected that host mineral. The lack of observed hydrous crystalline phases in the $<150\text{-}\mu\text{m}$ fraction (15) implies that $\text{H}_2\text{O}/\text{OH}$ is derived from the amorphous component. H_2O concentrations in the amorphous component are estimated to be 3 to 6 wt % H_2O .

Unlike the situation for H_2O , calculated abundances of carbonate inferred from CO_2 released, sulfate minerals from SO_2 , and oxychloride compounds (e.g., chlorate or perchlorate) from O_2 would all be at or below the detection limits of CheMin, affirming the complementarity of SAM and CheMin on Curiosity. The data do not allow specific determination of whether host materials for these evolved gases exist as crystalline phases

at abundances less than the 1 to 2% detectable by CheMin, or whether these volatiles are also hosted in amorphous materials in the $<150\text{-}\mu\text{m}$ fraction. However, the release temperatures of the gases suggest fine-grained and/or poorly crystalline materials as the hosts, as discussed below.

The CO_2 released from all four Rocknest runs comprises two major peaks, at $\sim 400^\circ$ and $\sim 510^\circ\text{C}$, and a lower-temperature shoulder, which can be fit as two discrete releases at $\sim 225^\circ$ and $\sim 295^\circ\text{C}$ (Fig. 3). The two major CO_2 peaks together comprise $>70\%$ of the CO_2 released. The highest-temperature CO_2 release is consistent with the thermal decomposition of siderite (19). If this peak is due entirely to siderite decomposition, it would imply ~ 1 wt % siderite in the Rocknest $<150\text{-}\mu\text{m}$ fraction. A second possibility is that this release evolved from the thermal decompo-

sition of nanophase magnesite, because nanophase carbonates decompose at temperatures at least 100°C lower than 2- to $50\text{-}\mu\text{m}$ -sized particles (17, 20). Calcite is not a likely candidate because its decomposition begins at 685°C , a temperature substantially higher than that of the vast majority of CO_2 released from the Rocknest $<150\text{-}\mu\text{m}$ fraction. A third possibility is that the two major CO_2 peaks correspond to CO_2 chemically evolved from two mineral phases, such as siderite and magnesite, by reaction with HCl (18), which is observed in the Rocknest EGA (Fig. 1B), likely from decomposition of a perchlorate salt (see below). Most likely, all three factors (grain size, mineralogy, and reaction with HCl) contribute to the two major CO_2 peaks.

The concurrent evolution of CO_2 and O_2 from Rocknest suggests that organic carbon (i.e., C con-

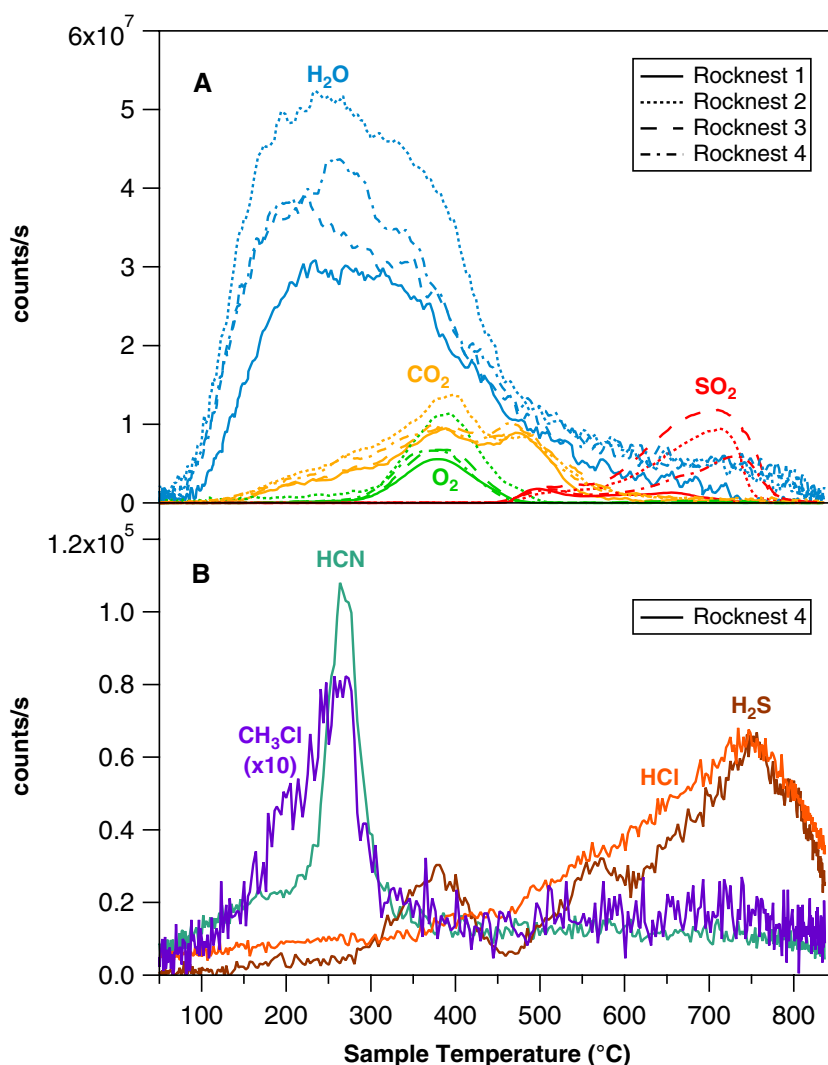


Fig. 1. Gases released from heated Rocknest aliquots. Relative abundance of molecular ions diagnostic of specific gases evolved over the 75° to 835°C pyrolysis temperature ramp. (A) The four most abundant gases evolved from the four Rocknest portions delivered to SAM. Major molecular ions that saturated the QMS detector were estimated on the basis of other isotopologs of that species. (B) Traces for m/z 27, 34, 36, and 52, reflecting four minor gases from the Rocknest run 4. Gas species that constitute the greatest input to the traces are labeled (27 = HCN , 34 = H_2S , 36 = HCl , and 52 = CH_3Cl), as are any scaling factors used. Minor contributions from other species are possible (e.g., the low-temperature peak of the “ H_2S ” trace reflects a contribution from $^{16}\text{O}^{18}\text{O}$).

tained in molecules having C, H, O, N, and/or S) oxidized within SAM is another potential CO₂ source. Such reduced carbon might be indigenous to Mars, delivered from space in the form of interplanetary dust particles and micrometeorites, or part of the instrument background. Molecular fragments from a reagent carried to Mars for use in a SAM wet chemistry experiment, MTBSTFA (*N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide), have been identified in both empty-cup blank and Rocknest runs. A small fraction of CO₂ (<10% of the total CO₂ observed) from combustion of these organics is suggested by the amount of the most abundant MTBSTFA-related products, mono- and bi-silylated H₂O (*tert*-butyldimethylsilanol and 1,3-

bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane, respectively). These sources are discussed below in conjunction with $\delta^{13}\text{C}$ measurements and organic molecular analyses.

Although the intensity and shape of traces attributable to SO₂ vary between the Rocknest samples, overall, the EGA traces indicate that SO₂ evolves from ~450° to 800°C. Two main peaks are observed, at ~500° to 550°C and ~700° to 750°C (Fig. 1). Possible sources of the evolved SO₂ include the thermal decomposition of sulfates and/or sulfites, oxidation of sulfides, and S adsorbed onto particle surfaces, which can persist to relatively high temperatures (21). Laboratory EGA under SAM-like conditions shows that iron sulfates produce

SO₂ at temperatures consistent with Rocknest observations. Mg- and Ca-sulfates, including the anhydrite observed in Rocknest <150- μm fraction by CheMin (15), have SO₂ evolution temperatures too high to explain the observed SO₂. The high-temperature tail of O₂ peak at ~460°C is coincident with the initial rise of SO₂. This observation and SAM EGA detections of small amounts of H₂S, OCS, and CS₂ evolved at temperatures close to the higher-temperature SO₂ release (Fig. 1) support the hypothesis that oxidative reactions of reduced sulfur phases during heating also contributed to the evolved SO₂.

The onset of release of O₂ correlates with the release of chlorinated hydrocarbons (Fig. 1), suggesting that an oxychloride compound, such as a chlorate or perchlorate, is the source of the oxygen and chlorinated volatiles. Laboratory evaluation of various perchlorates and chlorates has not identified an unequivocal match to the SAM Rocknest data, but Ca-perchlorate provides the most reasonable match, with Fe- and Mg-bearing perchlorate, various chlorates, and mixtures with other minerals that may affect decomposition temperatures (22–24) as other possibilities.

The likely detection of an oxychloride compound by SAM is consistent with perchlorate observed in samples analyzed by the Wet Chemistry Laboratory (WCL) and the Thermal and Evolved Gas Analyzer (TEGA) instrument on the Phoenix lander (25), which observed a similar O₂ release during analysis of a soil sample. On the basis of WCL results, Phoenix soils were calculated to contain 0.4 to 0.6 wt % ClO₄[−] (25). If all of the oxygen detected by SAM resulted from perchlorate decomposition, the estimated ClO₄[−] abundance in the Rocknest <150- μm fraction (Table 2) would be comparable to the abundances observed by Phoenix. This abundance does not account for all of the chlorine detected by Curiosity's Alpha Particle X-ray Spectrometer (APXS) (14), implying the presence of other chlorine-bearing species at Rocknest.

Chlorine has been detected in every soil ever analyzed on Mars—in situ at the equatorial and mid-latitude sites of the two Viking landers (2) and from equator to mid-latitude by remote sensing from Mars Odyssey spacecraft (26). The process of perchlorate formation is believed to start with the oxidation of chlorine in gas-phase reactions in the atmosphere (27), various chlorine oxides produced by energetic electrons from galactic cosmic-ray interaction with the surface ice (28), heterogeneous mineral-catalyzed photo-oxidation of surface chlorides (29), or on airborne dust. The global presence of chlorine, and the detection of perchlorate in fines at two very different locations (Phoenix and Curiosity landing sites), support the hypothesis that perchlorates are globally distributed in the regolith of Mars. Perchlorates can be a sensitive marker of past climate and a potential terminal electron acceptor for martian biota. They may also form liquid brines under current martian conditions and contribute to the oxidation and transformation of martian

Table 2. Abundance of major species released upon heating of Rocknest as measured with the SAM QMS. Errors reported for molar abundances are the 2 σ SD from the mean of calculations done with different *m/z* values for the same species. Weight % values were calculated with an estimated sample mass of 50 $\pm$ 8 mg (2 σ), with errors propagated including the uncertainty in molar abundance (14).

Molar abundances (μmol)				
	Run 1	Run 2	Run 3	Run 4
CO ₂	8.3 $\pm$ 2.0	10.8 $\pm$ 2.6	10.1 $\pm$ 2.4	10.4 $\pm$ 2.5
SO ₂	2.9 $\pm$ 0.2	13.7 $\pm$ 1.9	21.7 $\pm$ 2.9	10.5 $\pm$ 1.4
H ₂ O	43.3 $\pm$ 10.7	66.5 $\pm$ 16.2	54.5 $\pm$ 9.9	55.9 $\pm$ 11.9
O ₂	3.0 $\pm$ 0.4	5.1 $\pm$ 0.6	3.7 $\pm$ 0.4	3.7 $\pm$ 0.5
Sample weight %				
	Run 1	Run 2	Run 3	Run 4
CO ₂	0.7 $\pm$ 0.2	1.0 $\pm$ 0.3	0.9 $\pm$ 0.3	0.9 $\pm$ 0.3
SO ₃ equiv.	0.5 $\pm$ 0.1	2.2 $\pm$ 0.5	3.5 $\pm$ 0.7	1.7 $\pm$ 0.3
H ₂ O	1.6 $\pm$ 0.5	2.4 $\pm$ 0.7	2.0 $\pm$ 0.5	2.0 $\pm$ 0.5
ClO ₄ equiv.	0.3 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1

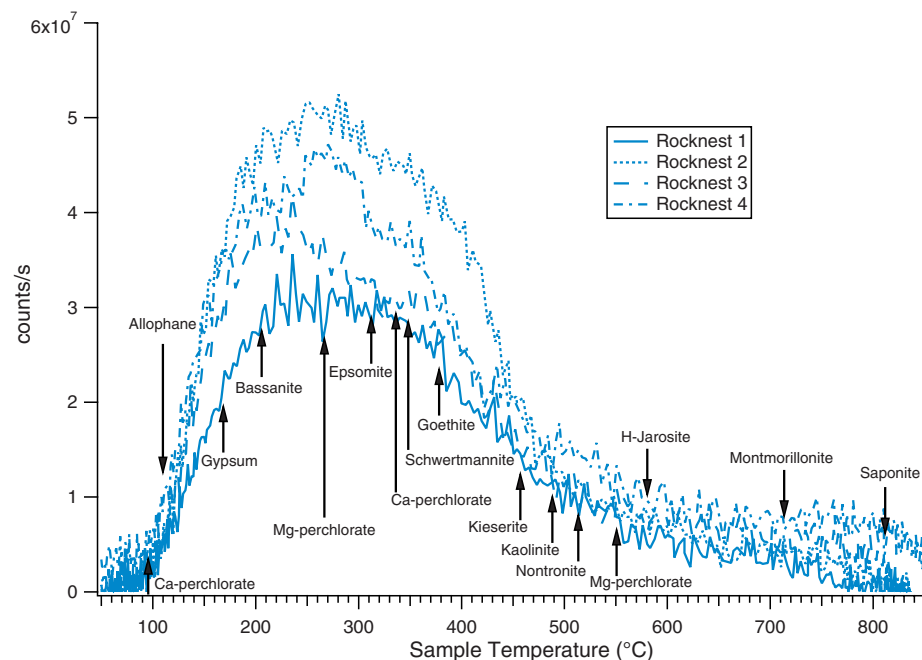


Fig. 2. Water release from Rocknest compared to laboratory measurements of mineral breakdown. Water release versus temperature for Rocknest <150- μm fraction measured by the SAM QMS. Arrows indicate temperatures of water-release peaks determined by laboratory analysis for select hydrous minerals phases under conditions similar to that in SAM (17).

organic matter when exposed to ionizing radiation at or near the surface or during analytical processing. Thus, a widespread presence of perchlorate salts, spatially and temporally, would have an important bearing on understanding habitability, organic matter preservation potential, and organic biosignature detection on Mars.

Isotopes

The results of the TLS isotopic analyses at Rocknest are summarized in Table 3. The strategy for the different temperature ranges of evolved gas sent to the TLS was developed with the EGA data to iteratively design experiments that selectively focused on various gas releases. For example, run 3 captured the bulk of the H₂O peak, and run 4 focused on the first of the two large CO₂ peaks. The EGA data were also used to constrain the isotopic composition of C in CO₂ and S in SO₂.

Hydrogen in all Rocknest samples is highly enriched in deuterium compared to terrestrial materials (Fig. 4), with δD values ranging from $+3900$ to $+7000$ per mil (‰). Run 3 should be most representative of the “bulk” of the water in Rocknest, with a value of $+7000$ ‰. However, significant variation in the δD value with temperature is observed, with the lower-temperature cut having the highest δD value and the highest-temperature cut having the lowest.

The δD values measured in the Rocknest $<150\text{-}\mu\text{m}$ fraction are consistent with the SAM TLS measurements of water in the martian atmosphere taken before Rocknest, which show a δD value of $+5000 \pm 1000$ ‰ (30). In addition, the Rocknest δD values are within the range of values observed by remote-sensing analysis of the martian atmosphere (31), where telescopic measurements from Earth have previously suggested a reservoir enriched in D by a factor of ~ 5 over terrestrial values. The D-enriched values in a martian soil are also consistent with D-enriched H₂O observed in both bulk (32) and single grains (33) in martian meteorites.

The close match between the δD values from H₂O in both atmospheric gas and Rocknest suggests that the H₂O-rich phases in the amorphous material were formed either in direct contact with the atmosphere or through interaction with volatiles derived from it. The variation of δD value with temperature may either record long-term variation of D/H through time or represent seasonal variations reflecting changes in the water cycle. It is likely that the water evolved at the lowest temperatures represents water in active exchange with the present atmosphere, whereas the higher-temperature releases could represent water from a more ancient time. Telescopic measurements suggest that there could be large variations in atmospheric δD value with water content of the atmosphere and season (31), and such variations may be reflected in the Rocknest results.

Like hydrogen in H₂O, ¹³C-enriched CO₂ has also been observed in the atmosphere at Gale

crater with SAM TLS (30) and QMS (34), with an average $\delta^{13}\text{C}$ value measured to date of $\sim +46$ ‰. Unlike hydrogen, however, the CO₂-bearing phases in Rocknest soil do not fully reflect this ¹³C-enriched atmospheric value. Rather, $\delta^{13}\text{C}$ values of CO₂ evolved from Rocknest and analyzed by TLS range from -6 to $+20$ ‰ (Table 3), and estimates of $\delta^{13}\text{C}$ over the two major CO₂ peaks using QMS data average $\sim +18 \pm 10$ ‰, consistent with the TLS results. These values overlap with $\delta^{13}\text{C}$ values from both carbonates and refractory/reduced carbon in martian meteorites (Fig. 5). Consistent with the above discussion of several possible CO₂ sources in SAM analyses of Rocknest, the $\delta^{13}\text{C}$ compositions likely reflect mixing of multiple carbon sources. The concurrent evolution of CO₂ and O₂ from Rocknest suggests that partial combustion of reduced carbon could contribute to evolved CO₂. $\delta^{13}\text{C}$ associated with the CO₂ release between 250° and 450°C might reflect some contribution from this combusted carbon. Previous studies of martian meteorites have shown that reduced carbon is present either as an indigenous component or from exogenous meteoritic input (8, 10–12).

The Rocknest $\delta^{13}\text{C}$ values suggest a hint of ¹³C enrichment, consistent with $\delta^{13}\text{C}$ values observed in martian meteorite carbonates. Specifically, the data from run 4, which most closely capture the largest CO₂ peak, has a $\delta^{13}\text{C}$ value of $+20 \pm 10$ ‰, which is similar to carbonate measured in the Nakhla meteorite (35). This value is lower than would be expected for carbonate formed from the modern atmosphere as measured by SAM TLS (30). It is possible that this CO₂ release is a mixture of carbonate-derived CO₂ with a high $\delta^{13}\text{C}$ value and CO₂ depleted in ¹³C and thus does not reflect the true carbon isotopic composition of the carbonate. It is also possible that the carbonate does have low $\delta^{13}\text{C}$ values as observed in some of the martian meteorites, suggesting that the atmosphere has changed through time (36). Overall, the data support a minor amount of carbonate in martian soil derived from atmosphere interaction with only transient water (37).

The sulfur isotopic composition of SO₂ released during run 4 was determined from QMS data at a mass-to-charge ratio (m/z) of 64, 65, and 66. The Rocknest $<150\text{-}\mu\text{m}$ fraction, including analyses of both of the major SO₂ evolution peaks,

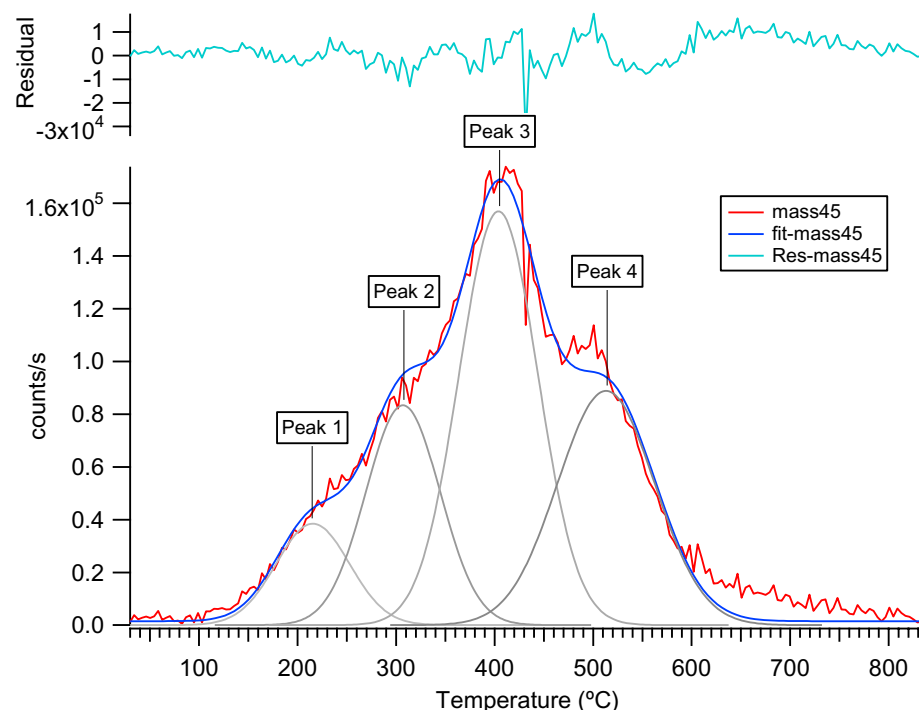


Fig. 3. Deconvolution of CO₂ release from Rocknest. Rocknest run 2 CO₂ (mass 45) versus temperature (red). Gray peaks are Gaussian fits to overall CO₂ release that sum to mass 45 fit (blue line). CO₂ fractions in each of the four peaks are 0.07, 0.22, 0.41, and 0.30, respectively.

Table 3. Isotopic composition of volatiles released upon heating of Rocknest as measured with the SAM TLS. Blank cup corrections have been applied as described in materials and methods.

Rocknest run	T range sampled (°C)	$\delta^{13}\text{C}$ in CO ₂ (‰)	δD in H ₂ O (‰)
Run 3	234–425	-6 ± 14	7010 ± 66
Run 4	350–443	20 ± 10	4250 ± 60
Run 2	440–601	3 ± 9	3870 ± 60

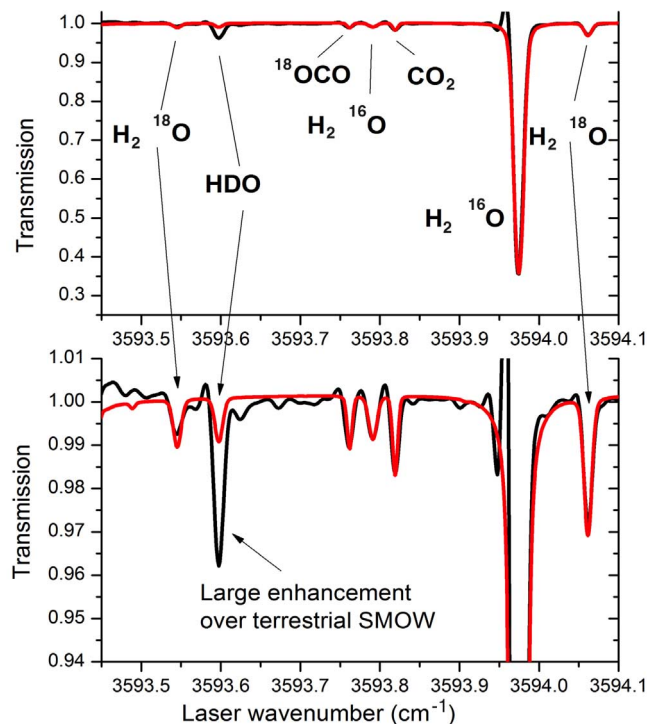
have $\delta^{34}\text{S}_{\text{VCDT}}$ of $0 \pm 10\%$, consistent with sulfur isotopic compositions measured in martian meteorites (38, 39).

Organic Matter

Chlorohydrocarbons comprising chloromethane (CH_3Cl), dichloromethane (CH_2Cl_2), trichloromethane (CHCl_3), and chloromethylpropene ($\text{C}_4\text{H}_7\text{Cl}$) were detected during SAM GC-MS analyses (Fig. 6 and Table 4). Chloromethanes detected by SAM

in runs 1, 2, and 4 were at $\sim$ nanomole levels and above SAM background. Run 3 produced lower abundances of chloromethanes (typically observed at $<300^\circ\text{C}$) because only a high-temperature cut of evolved gases were transferred to the GC. Minor amounts of HCN, CH_3Cl , CH_2Cl_2 , and CHCl_3 are also observed in SAM EGA data (Fig. 1B). The abundance of these species is more than two orders of magnitude lower than that of the most abundant volatile released— H_2O .

Fig. 4. Tunable laser spectrometer data showing hydrogen isotope enhancement in Rocknest. Section of a single spectrum (60 s integration) downloaded from Curiosity (black) for the Rocknest 3 sample run, showing large HDO line depth compared to calculated HITRAN spectrum (red) based on terrestrial SMOW water isotope ratios. The HDO line is ~ 4 times the depth of that predicted for SMOW, so that the D/H ratio is ~ 8 times that of SMOW, corresponding to a δD value of $\sim 7000\text{‰}$, as reported.



The abundances measured by SAM are higher than the picomole levels (up to 40 parts per billion) for chloromethane and dichloromethane previously measured by the Viking pyrolysis gas chromatography–mass spectrometry (GC-MS) instruments after heating the samples of scooped fines up to 500°C (13). Biemann *et al.* (13) attribute the Viking results to chlorohydrocarbons derived from cleaning solvents used on the instrument hardware, not from the martian samples themselves. Recently, Navarro-González *et al.* (40) suggested that these chlorohydrocarbons may have formed by oxidation of indigenous organic matter during pyrolysis of the soil in the presence of perchlorates, but Biemann and Bada (41) disagree with this conclusion.

The absence of detectable chlorohydrocarbons in the SAM blank run indicates that the chlorohydrocarbons measured at Rocknest are not directly attributable to the SAM instrument background signal. However, the associated release of chloromethanes, O_2 , and HCl strongly suggests that these chlorohydrocarbons are being produced within SAM by chlorination reactions involving an oxychloride compound in the Rocknest $<150\text{-}\mu\text{m}$ fraction and an organic carbon precursor (23). Three sources for the organic carbon of this reaction are possible: (i) terrestrial sources within the SAM instrument or the Curiosity sample chain; (ii) exogenous carbon in the martian surface materials derived from infalling meteoritic carbon; and (iii) martian indigenous organic matter. A feasible explanation involves terrestrial carbon derived from the MTBSTFA, whose reaction products were identified in both the blank and soil EGA and GC analyses. On the basis of laboratory pyrolysis GC-MS experiments, pyrolytic reaction of martian Cl with organic carbon from MTBSTFA in SAM can explain the presence of the chloromethanes and chloromethylpropene detected by SAM. However, we cannot rule out the possibility that traces of organic carbon of either martian or exogenous origin contributed to some of the chlorohydrocarbons measured by SAM at Rocknest.

Overall, SAM analyses indicate that martian fines contain a number of materials with bound volatiles that can be released upon heating. These volatile-bearing materials are likely very fine-grained and associated with the amorphous component of martian regolith. The fines could be a good source of water, CO_2 , and other volatiles to be leveraged by future human explorers on Mars. Isotopic compositions support an atmospheric source of the water and possibly CO_2 , consistent with previously proposed formation mechanisms for carbonate and perchlorate in the fines that involve interaction with the atmosphere. Although martian organic matter was not definitively detected, the presence of materials that produce substantial amounts of oxygen upon heating suggests that detection of such compounds in martian soils will be difficult with pyrolysis techniques. The fines on Mars reveal a complex history, reflecting global, regional, and local-scale processes.

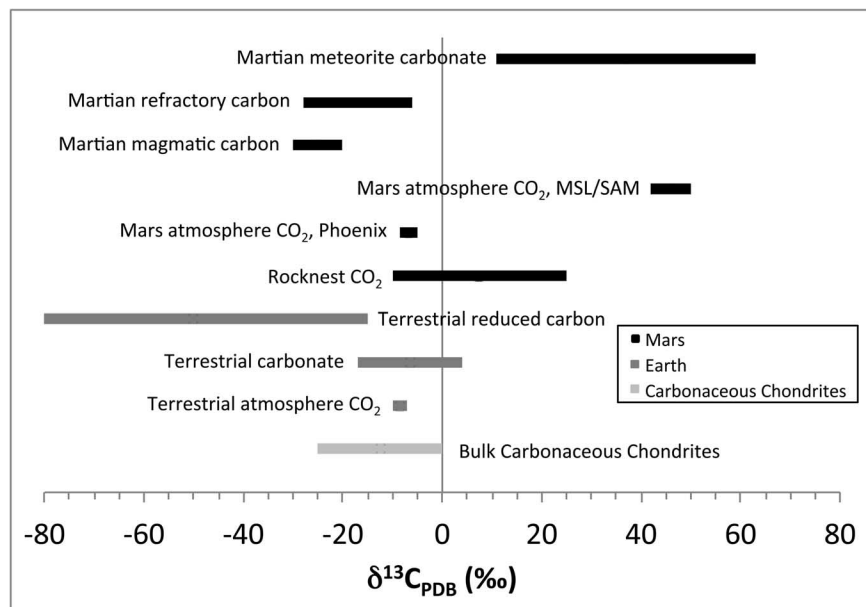


Fig. 5. Carbon isotopes in relevant solar system reservoirs. Carbon isotopic composition of materials from Mars (44–46), Earth (47), and carbonaceous chondrite meteorites (48) for comparison the values measured in Rocknest and the martian atmosphere (30) by the Mars Curiosity Rover.

Materials and Methods

SAM Instrument Overview and Operations

The SAM instrument suite supports the MSL mission and sits inside the Curiosity rover at Gale crater on Mars. The SAM instruments are a QMS, a TLS, and a six-column GC with thermal conductivity detectors (TCDs) (7). These three instruments share a solid sample- and gas-processing system to generate complementary compositional and isotopic observations on each sample delivered by the rover's Sample Acquisition, Sample Processing and Handling (SA/SPaH) hardware to a SAM solid sample inlet tube or ingested directly through a gas inlet. Before each analysis, the oven for solid samples, gas-processing system, and instruments are purged with helium and heated to release any residual volatiles in the system to, in effect, precondition and clean SAM.

Scooped, sieved <150- μm -particle-size fraction, and portioned (<76 mm³) sediments of the Rocknest aeolian drift were heated to thermally evolve gases for processing and analysis. These volatiles are the result of the following processes often happening concurrently: (i) desorption of surface-adsorbed volatiles and anions, (ii) mineral thermal decomposition, and (iii) thermo-

chemical reactions among chemical components (18). When organic materials are present in solid samples, they might be desorbed at low temperatures (usually below 320°C), as is the case for small individual molecules; undergo pyrolysis (i.e., thermal bond cleavage) at higher temperatures; or contribute to thermochemical reactions (at all temperatures) (42).

SAM performs EGA with the QMS and isotope measurements of evolved gases with both the QMS and the TLS, with the latter being sensitive to CO₂, water, and methane (methane detection capability was not used during Rocknest runs). Organic analyses can be performed with the QMS alone or when it is coupled to the GC. SAM heated each Rocknest sample to ~835°C at a rate of 35°C/min with a He carrier gas flow rate of ~0.77 standard cm³ per minute and at an oven pressure of ~25 mbar. The SAM QMS analyzed abundances of gases across the entire temperature range, while selected temperature ranges of the evolved gases in each run were sent to the TLS and GC for analysis (Table 1). For each portion ingested by SAM (called runs 1 through 4), the gases evolved across the selected range of temperatures were accumulated inside the TLS Herriott cell, where hydrogen isotopes in water

and carbon isotopes in CO₂ were analyzed in the bulk gases. Evolved gases from a different selected temperature range sent to the GC were first captured on the hydrocarbon trap held at 5°C. The trap was subsequently heated to ~300°C under He flow, and the desorbed gases were sent to a GC channel (composed of an injection trap, MXT-CLP column, and TCD) suited for the analysis and separation of volatile organic compounds. The TCD and QMS provide detection and identification of the chemical molecules eluted from the GC.

Solid Sample Analysis Details

At Rocknest, fines from scoop #5 were delivered to SAM four times and placed in separate quartz-glass cups. The sample in each cup was flushed with pure helium (99.999%) at ~25 mbar at ~0.77 standard cm³ per minute and heated at ~75°C for 15 min to release water adsorbed on mineral surfaces and minimize saturating the system with excess water. This early gas release was directly measured by the QMS. Each cup was heated in SAM oven #1 at a rate of ~35°C min⁻¹ to ~835°C, where the final temperature varied slightly due to different ambient Mars environmental conditions. All evolved gases from the 75° to 835°C range were sampled by the QMS

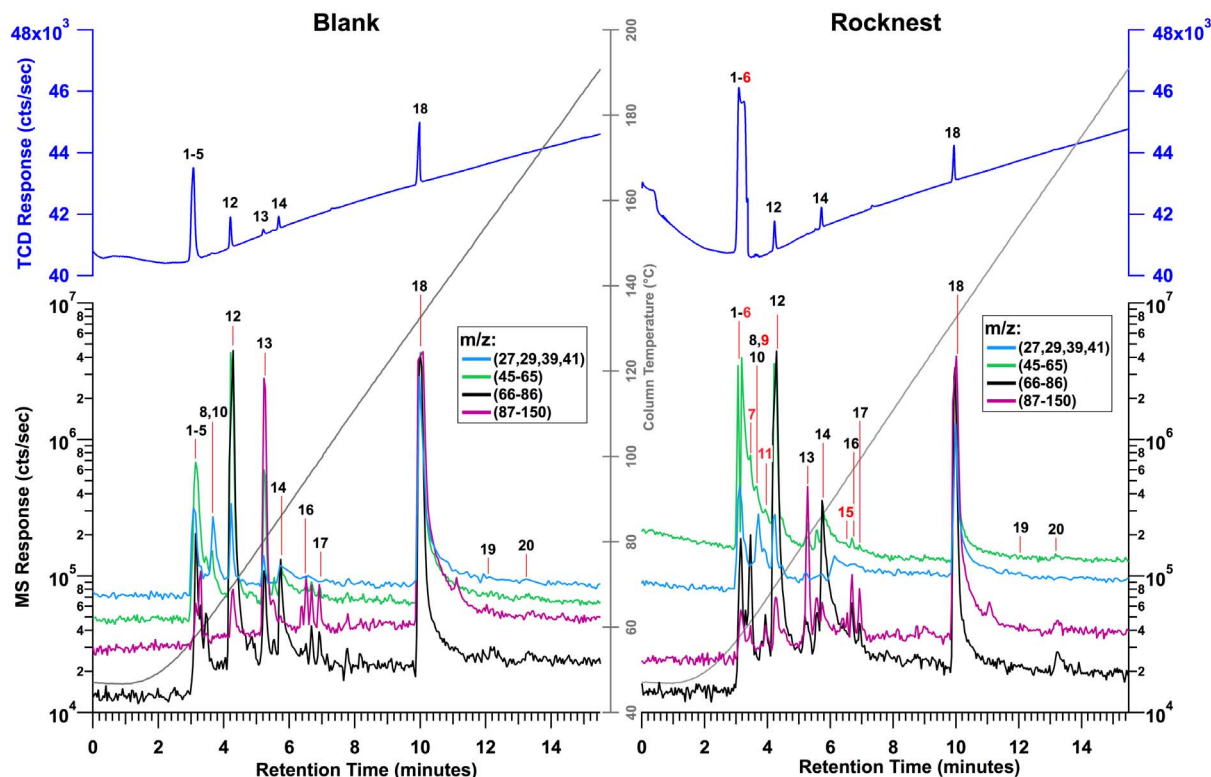


Fig. 6. Blank run and Rocknest gas chromatograph data. SAM gas chromatograph separation of volatile compounds released during the pre-Rocknest blank (left) and Rocknest run 1 (right). See materials and methods for analytical protocols. The top plots in blue show the relative intensity of the TCD signal versus GC retention time. The traces shown on the bottom plot represent peak intensities of four different scans over the specified *m/z* ranges in counts per second (cps) versus GC retention time. Key compounds (numbered) were identified by comparison to National Institute of Standards and Technology mass spectral references. The following peaks (marked in red in

the Rocknest figure) were identified above measured background levels: 2, carbon dioxide; 3, sulfur dioxide; 4, hydrogen cyanide; 5, hydrogen sulfide; 6, chloromethane; 7, dichloromethane; 9, trichloromethane; 11, chloromethylpropene; 15, chlorobenzene. The following peaks are consistent with measured background levels: 1, carbon monoxide; 8, acetone; 10, acetonitrile; 12, benzene; 13, toluene; 14, *tert*-butyldimethylsilanol; 16, phenylethyne; 17, styrene; 18, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane; 19, trimethylsilylborate; and 20, biphenyl. See Table 4 for discussion of possible origins of each peak.

through a capillary flow restrictor. This experiment is referred to as evolved gas analysis and produced data in the form of pyrograms for individual ions defined by their m/z ratios. The main split of evolved gas was then passed through gas manifolds heated at 135°C to either the hydrocarbon trap for GC analysis or the TLS Herriot cell for isotopic and mixing-ratio measurements of H₂O, CO₂, or O₂, or vented to Mars. For each analytical run, different temperature cuts of gas were selected to go to the GC or TLS, but in no case was the gas sent to both at the same time. Temperature cuts for the GC and TLS are listed in Table 1.

Figures 2 and 3 show detailed analyses of the H₂O and CO₂ releases, respectively. The plot of H₂O release in Fig. 2 is generated with the QMS data from m/z 20, because the molecular ion for H₂O (m/z 18) is saturated in these runs. The release temperatures of various hydrous mineral phases marked on the plot are derived from laboratory measurements performed under conditions similar to those for the EGA in SAM. These were typically determined for single minerals, and mineral mixtures and grain-size effects can change these values. Nonetheless, the broad H₂O release peak is not clearly indicative of any one mineral phase. Figure 3 shows evolved CO₂ (m/z 45) as a function of temperature for Rocknest run #2, for which four discrete peaks can be fit to Gaussian peak shape to model the summed CO₂ release. The integrated areas for the fitted

peaks are used to quantify the contributions from each release event to the total abundance of evolved CO₂. Although it is not possible to assign definitively specific species to each of the four peaks, oxidized organics (terrestrial or martian) and several types are carbonate are discussed in the text as likely contributing to the CO₂ peaks, especially the two major peaks.

Methods for Molar Abundance Calculations

Molar abundances were primarily computed by referencing measurements on Mars to pre-launch SAM calibration runs of quantified samples of calcite (CaCO₃) and a hydrated iron sulfate (FeSO₄·4H₂O) (6). A calibration factor [counts per second (cps)/mol] was determined for the relevant m/z value in laboratory standard runs by integrating under the evolved gas curve and dividing by the number of moles evolved from the sample, assuming complete decomposition.

Under nominal SAM operating conditions, the most abundant ion of major species (e.g., m/z 18 for H₂O) often saturates the detector. Given a fixed detector range, this makes the instrument more sensitive to low-abundance materials. To quantify amounts with high abundances, doubly ionized molecules, ion fragments, and isotopes were used to calculate evolved gas abundances. For example, m/z 44 (CO₂⁺) saturated the detector for most of the Rocknest runs because the amount of CO₂ evolved exceeded detector lim-

its. To quantify the abundance of CO₂, m/z 12 (C⁺), m/z 22 (CO₂²⁺), and m/z 45 and 46 (isotopologs of CO₂) were used instead of m/z 44. The number of moles of CO₂ evolved from Rocknest samples was determined by taking an average of the total areas calculated for each m/z listed above. The error was calculated as 2σ SD from the mean. For H₂O, m/z 19 and 20 were used because m/z 17 and 18 were saturated, in both Rocknest and earlier laboratory calibration runs. To calculate SO₂ abundances, m/z 66 and 50 were used (isotopologs of SO₂ and SO) because m/z 64 and 48 saturated in calibration runs.

There are two additional complications encountered when calculating H₂O abundances. First, FeSO₄·4H₂O begins to lose H₂O as soon it is exposed to lower pressures, which is before the QMS begins monitoring gas evolution. Fortunately, there is a very distinct and repeatable H₂O release at slightly higher temperatures (~200°C) with a measurable mass loss. This H₂O release was used to calibrate Rocknest data. Second, Mars has a much higher D/H ratio than Earth, which can affect peak integration values, especially for m/z 19 (HDO). We corrected for this effect when calculating the water abundances on Mars using QMS data during EGA. The differences in the δ¹⁸O, δ¹³C, and δ³⁴S isotopic values between Earth and Mars are small compared to the other uncertainties involved in these abundance calculations and, therefore, were not included.

Oxygen abundance values were calculated in a slightly different way because none of the minerals run during prelaunch testing released O₂. However, a separate prelaunch characterization run was done with an equimolar gas mix of O₂, CO₂, Ar, and N₂. These data were used to determine relative calibration factors for the major atmospheric species, as discussed in Mahaffy *et al.* (34). Such calibration factors yield a value for relative ionization rates for O₂ and CO₂ at equivalent abundances, which were applied to the EGA data to determine calibration factors for O₂ in cps/mol.

Isotope Data Reporting Convention

All isotope results are presented in standard delta notation (δD, δ¹³C, δ³⁴S) with respect to Vienna standard mean ocean water (VSMOW) for hydrogen, Vienna Pee Dee belemnite (VPDB) for carbon, and Vienna Cañon Diablo troilite (VCDT) for sulfur. Here, δ(‰) = [$R_{\text{meas}}/R_{\text{std}} - 1$] × 1000, where R_{meas} is the measured isotope ratio (heavy/light), and R_{std} is the ratio of the relevant reference standard.

TLS Operational Conditions and Data Reduction

TLS is a two-channel tunable laser spectrometer that uses direct and second harmonic detection of infrared laser light absorbed after multipassing a sample cell. For the results reported here, the sample cell path length is 43 passes of a ~19.5-cm cell length, or 840 cm. TLS scans over individual rovibrational lines in two spectral regions near 2.78 μm; one centered at 3590 cm⁻¹ for

Table 4. Inorganic and organic volatile species detected by the SAM GC-MS upon heating of Rocknest and their possible origins. MTBSTFA (*N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide) and DMF (dimethylformamide) are both carried within SAM for future derivatization experiments. Tenax TA is a porous polymer adsorbent resin used to concentrate organic compounds on the SAM hydrocarbon traps. Those sources that are known to be terrestrial in origin are shown in italics. Compounds in bold are observed above measured background levels.

Peak no. from Fig. 6	Compound	Possible origin(s)
1	Carbon monoxide	Unknown
2	Carbon dioxide	Martian carbonates/carbon?, <i>MTBSTFA or DMF</i>
3	Sulfur dioxide	Martian S-bearing minerals
4	Hydrogen cyanide	<i>MTBSTFA</i> + perchlorate or high-T martian source?
5	Hydrogen sulfide	Product of S-bearing minerals
6	Chloromethane	<i>MTBSTFA</i> or martian carbon? + perchlorates
7	Dichloromethane	<i>MTBSTFA</i> or martian carbon? + perchlorates
8	Acetone	<i>MTBSTFA or DMF</i>
9	Trichloromethane	<i>MTBSTFA</i> or martian carbon? + perchlorates
10	Acetonitrile	<i>MTBSTFA or DMF</i>
11	Chloromethylpropene	<i>MTBSTFA</i> + perchlorates
12	Benzene	<i>Tenax TA</i>
13	Toluene	<i>Tenax TA</i>
14	<i>tert</i> -Butyldimethylsilanol	<i>MTBSTFA</i> + water
15	Chlorobenzene	HCl + Cl ₂ + <i>Tenax TA</i>
16	Phenylethyne	<i>Tenax TA</i>
17	Styrene	<i>Tenax TA</i>
18	1,3-bis(1,1-dimethylethyl)- 1,1,3,3-tetramethyldisiloxane	<i>MTBSTFA</i> + water
19	Trimethylsilylborate	<i>MTBSTFA</i> + glass beads
20	Biphenyl	<i>Tenax TA</i>

CO₂ isotopes, and a second centered at 3594 cm⁻¹ for both CO₂ and H₂O isotopes. The lines used in both regions have no discernable interferences. In the 3594-cm⁻¹ region, the CO₂ and H₂O lines used interleave across the spectrum without interference, allowing determination of isotope ratios across widely varying CO₂ and H₂O abundances in both atmospheric and evolved gas experiments. For carbon isotopes, the values given are the weighted means of two separate pairs of lines, one pair from each region. Further data-processing details and calibration are described in the supplementary materials accompanying Webster *et al.* (30). Figure 4 is a good example of a TLS flight spectrum used for isotope ratio measurement, showing the large enhancement of the HDO line over that expected (HITRAN database) for terrestrial water.

The TLS sample cell (Herriott cell) is first pumped out by using the SAM turbomolecular pump with empty cell pressures of CO₂ and H₂O that are insignificant compared to either EGA or “blank cup” runs. At some predetermined time during either the four EGA or single blank cup runs, temperature cuts of evolved gas are sent to the TLS (Table 1), where they produced Herriott cell pressures of 4 to 9 mbar of principally helium, with evolved water and carbon dioxide as minor components. Before the Rocknest EGA runs reported here, a blank cup run was conducted under the identical conditions (He flow, temperature cut, pressure, etc.) but without solid sample in the pyrolysis oven. Resulting signals (abundances) for CO₂ and H₂O were not large compared to the Rocknest abundance values, and isotope values are similar to those of the samples, but nonetheless it is appropriate to make a correction. This correction was weighted by the relative abundance of the gas of interest. Specifically, the H₂O abundances in the blank were ~3% of the total water measured, and the CO₂ abundances were ~5 to 10% of the total CO₂ measured in Rocknest aliquots. Blank cup values for measured δ¹³C in CO₂ was -80‰ and δD in H₂O was 3880‰. The TLS measured results are therefore the combination of an underlying background (blank cup) contribution and the Rocknest sample contribution in proportions dependent on the relative abundances of water and carbon dioxide from each. Because the blank cup abundances of these gases is much smaller than those evolved from the Rocknest samples, the corrections to the measured isotope ratios are usually small. Given the small abundance of H₂O and CO₂ in the blank, the blank isotope values have relatively large uncertainties, and these are propagated through the correction calculation. The results given in Table 3 are the Rocknest sample isotope ratios after correction for the blank cup values.

QMS Isotope Value Calculations

Isotope ratios from QMS data obtained through EGA of solid samples are computed from the time-integrated signal at isotopologs for the compounds of interest. For CO₂, the ¹³C/¹²C ratio is

determined from *m/z* 45 and 46, with correction for oxygen contributions based on the most relevant TLS measurements of the oxygen isotopic composition in CO₂. The values of δ¹³C given in the main text were computed on the basis of an estimated δ¹⁸O value of -28‰, as determined by the TLS for CO₂ released during Rocknest 4, because the gas sample sent to TLS during this run was the most representative of the peak CO₂ release from Rocknest samples. The carbon isotopic composition of CO₂ during these runs cannot be calculated from the more typical pairs of *m/z* 12-13 or 44-45 because of interference from MTBSTFA background at *m/z* 13 and detector saturation at *m/z* 44.

For SO₂, the ³⁴S/³²S ratio is computed from *m/z* 64-66, with correction for oxygen contributions (assumed δ¹⁸O of 50 ± 5‰) based on TLS measurements of the oxygen isotopic composition of both CO₂ and H₂O in the martian atmosphere. For both CO₂ and SO₂, ¹⁷O contributions were estimated from the assumed δ¹⁸O on the basis of a Δ¹⁷O of 0.32‰, the average for martian silicates (43).

GC-TCD and GC-MS Operational Conditions

The SAM GC hydrocarbon trap consists of three layers: nonporous silica beads, Tenax TA adsorbant (porous 2,6-diphenylene oxide polymer resin), and Carbonsieve G adsorbant (graphitized carbon) (7). Gases were passed through the hydrocarbon trap that had been cooled to 5°C and selectively condensed onto glass beads or adsorbed on the basis of volatility, molecular size, and chemistry. Thermal desorption at 300°C for 4 min under He (0.5 standard cm³ per minute, 0.9 bar) released analytes from the hydrocarbon trap in the opposite direction. Analytes then collected on a Tenax TA injection trap of the GC. The injection trap was then flash heated to 300°C. All four Rocknest GC analyses used the MTX-CLP column (30-m length, 0.25-mm internal diameter, 0.25-μm film thickness), which has a polydimethylsiloxane with phenyl and cyanopropyl film (7) and is designed for separating mid-molecular weight hydrocarbons. The column temperature was programmed from 50° to 220°C at 10°C min⁻¹ and He carrier gas was held at a constant column inlet pressure of 0.9 bar. Gases eluting from the GC were nondestructively detected by the TCD and then ionized by electron impact at 70 eV in the QMS source, which fragmented molecules in a predictable fashion. The QMS scanned for ions in a *m/z* range of 2 to 535 using the Smart Scanning algorithm previously described (7). The GC-TCD result is shown as a single chromatogram for all detected molecules in which the retention time (*x* axis) is normalized to the GC retention time. GC-MS results are given as individual ion chromatograms and mass spectra for isolated peaks, in which the mass spectra were generated with a custom data-processing program.

The identification of the chlorohydrocarbons in the GC-MS data was based on the retention times and comparisons of the mass fragmentation

patterns to the NIST11 library. The abundances of the chlorohydrocarbon compounds (~10⁻² to 1 nmol) were determined by comparing the fitted peaks in the Rocknest data to those of known amounts of hexane measured during preflight calibration runs on SAM GC, corrected for differences in ionization efficiency (34). These abundances are also corrected for the gas fraction sent to the hydrocarbon trap with EGA data (Table 1).

EGA, GC-TCD, and GC-MS Background Measurements

Within SAM, there are several common sources of organic molecules that are typical of similar, nonflight instruments. These include (i) wet chemistry reagents used for derivatization (i.e., MTBSTFA and DMF) and thermochemolysis [i.e., tetramethylammonium hydroxide in methanol (TMAH)] and their breakdown products; (ii) the Tenax TA, a porous, 2,6-diphenylene oxide polymer resin adsorbant that slowly degrades with use into benzene, toluene, tropylium ion, biphenyl, and other single-aromatic ring structures; and (iii) the polymer films inside the capillary columns that promote selective molecular separation. The molecules from these sources are known and make up what is referred to as instrument background signal, which is expected to change over the course of the SAM instrument's lifetime and is continuously monitored by analysis of empty sample cup or “blanks.” Contributions of aromatics from the Tenax TA traps to TCD and QMS (post-GC) data have been determined on the basis of their absence in EGA data. The blank analysis run before Rocknest fines was the first analysis performed since prelaunch testing, and it showed the presence of DMF, MTBSTFA products, Tenax TA products, and terrestrial water (Table 4). TLS measurements of D/H in water indicated that further heating and purging of the system largely eliminated the terrestrial water.

References and Notes

1. J. Brückner, G. Dreibus, R. Rieder, H. Wanke, Refined data of Alpha Proton X-ray Spectrometer analyses of soils and rocks at the Mars Pathfinder site: Implications for surface chemistry. *J. Geophys. Res. Planets* **108**, 8094 (2003). doi: [10.1029/2003JE002060](https://doi.org/10.1029/2003JE002060)
2. B. C. Clark *et al.*, Chemical composition of Martian fines. *J. Geophys. Res.* **87**, 10059 (1982). doi: [10.1029/JB087iB12p10059](https://doi.org/10.1029/JB087iB12p10059)
3. R. Gellert *et al.*, Chemistry of rocks and soils in Gusev Crater from the alpha particle x-ray spectrometer. *Science* **305**, 829–832 (2004). doi: [10.1126/science.1099913](https://doi.org/10.1126/science.1099913); pmid: [15297665](https://pubmed.ncbi.nlm.nih.gov/15297665/)
4. H. Wänke, J. Brückner, G. Dreibus, R. Rieder, I. Ryabchikov, Chemical composition of rocks and soils at the pathfinder site. *Space Sci. Rev.* **96**, 317–330 (2001). doi: [10.1023/A:1011961725645](https://doi.org/10.1023/A:1011961725645)
5. A. S. Yen *et al.*, An integrated view of the chemistry and mineralogy of martian soils. *Nature* **436**, 49–54 (2005). doi: [10.1038/nature03637](https://doi.org/10.1038/nature03637); pmid: [16001059](https://pubmed.ncbi.nlm.nih.gov/16001059/)
6. S. R. Taylor, S. M. McLennan, *Planetary Crusts: Their Composition, Origin and Evolution* (Cambridge Univ. Press, Cambridge, 2009).
7. P. R. Mahaffy *et al.*, The Sample Analysis at Mars Investigation and Instrument Suite. *Space Sci. Rev.* **170**, 401–478 (2012). doi: [10.1007/s12124-012-9879-z](https://doi.org/10.1007/s12124-012-9879-z)

8. M. M. Grady, A. B. Verchovsky, I. P. Wright, Magmatic carbon in Martian meteorites: Attempts to constrain the carbon cycle on Mars. *Int. J. Astrobiol.* **3**, 117–124 (2004). doi: [10.1017/S1473550404002071](https://doi.org/10.1017/S1473550404002071)
9. A. Steele *et al.*, Comprehensive imaging and Raman spectroscopy of carbonate globules from Martian meteorite ALH 84001 and a terrestrial analogue from Svalbard. *Meteorit. Planet. Sci.* **42**, 1549–1566 (2007). doi: [10.1111/j.1945-5100.2007.tb00590.x](https://doi.org/10.1111/j.1945-5100.2007.tb00590.x)
10. A. Steele *et al.*, A reduced organic carbon component in martian basalts. *Science* **337**, 212–215 (2012). doi: [10.1126/science.1220715](https://doi.org/10.1126/science.1220715); pmid: [22628557](https://pubmed.ncbi.nlm.nih.gov/22628557/)
11. L. Becker, B. Popp, T. Rust, J. L. Bada, The origin of organic matter in the Martian meteorite ALH84001. *Adv. Space Res.* **24**, 477–488 (1999). doi: [10.1016/S0273-1177\(99\)00090-3](https://doi.org/10.1016/S0273-1177(99)00090-3); pmid: [11543335](https://pubmed.ncbi.nlm.nih.gov/11543335/)
12. M. A. Sephton *et al.*, High molecular weight organic matter in martian meteorites. *Planet. Space Sci.* **50**, 711–716 (2002). doi: [10.1016/S0032-0633\(02\)00053-3](https://doi.org/10.1016/S0032-0633(02)00053-3)
13. K. Biemann *et al.*, The search for organic substances and inorganic volatile compounds in the surface of Mars. *J. Geophys. Res.* **82**, 4641–4658 (1977). doi: [10.1029/J5082i028p04641](https://doi.org/10.1029/J5082i028p04641)
14. D. F. Blake *et al.*, Curiosity at Gale crater, Mars: Characterization and analysis of the Rocknest sand shadow. *Science* **341**, 1239505 (2013).
15. D. L. Bish *et al.*, X-ray diffraction results from Mars Science Laboratory: Mineralogy of Rocknest at Gale crater. *Science* **341**, 1238932 (2013).
16. R. C. Anderson *et al.*, Collecting samples in Gale Crater, Mars; an overview of the Mars Science Laboratory Sample Acquisition, Sample Processing and Handling System. *Space Sci. Rev.* **170**, 57–75 (2012). doi: [10.1007/s11214-012-9898-9](https://doi.org/10.1007/s11214-012-9898-9)
17. D. L. Bish, C. J. Duffy, in *Thermal Analysis in Clay Science*, J. W. Stucki, D. L. Bish, Eds. (Clay Minerals Society, Boulder, CO, 1990), vol. 3, pp. 95–189.
18. K. M. Cannon, B. Sutter, D. Ming, W. V. Boynton, R. C. Quinn, Perchlorate induced low temperature carbonate decomposition in the Mars Phoenix Thermal and Evolved Gas Analyzer (TEGA). *Geophys. Res. Lett.* **39**, L13203 (2012). doi: [10.1029/2012GL015952](https://doi.org/10.1029/2012GL015952)
19. B. Sutter *et al.*, The detection of carbonate in the martian soil at the Phoenix Landing site: A laboratory investigation and comparison with the Thermal and Evolved Gas Analyzer (TEGA) data. *Icarus* **218**, 290–296 (2012). doi: [10.1016/j.icarus.2011.12.002](https://doi.org/10.1016/j.icarus.2011.12.002)
20. H. V. Lauer Jr., P. D. Archer, B. Sutter, P. B. Niles, D. M. Ming, in *Lunar Planet. Sci.* **43** (2012), abstr. 2299.
21. B. C. Clark, A. K. Baird, Is the Martian lithosphere sulfur rich? *J. Geophys. Res.* **84**, 8395 (1979). doi: [10.1029/JB084iB14p08395](https://doi.org/10.1029/JB084iB14p08395)
22. R. Furuichi, T. Ishii, K. Kobayashi, Phenomenological study of the catalytic thermal decomposition of potassium perchlorate by iron(II) oxides with different preparing histories. *J. Therm. Anal.* **6**, 305–320 (1974). doi: [10.1007/BF01950062](https://doi.org/10.1007/BF01950062)
23. D. P. Glavin *et al.*, Evidence for perchlorates and the origin of chlorinated hydrocarbons detected by SAM at the Rocknest aeolian deposit in Gale Crater. *J. Geophys. Res. Planets* (2013). doi: [10.1002/jgre.20127](https://doi.org/10.1002/jgre.20127)
24. M. M. Markowitz, D. A. Boryta, The differential thermal analysis of perchlorates. VII. Catalytic decompositions of the alkali metal perchlorates by manganese dioxide. *J. Phys. Chem.* **69**, 1114–1123 (1965). doi: [10.1021/j100888a005](https://doi.org/10.1021/j100888a005)
25. M. H. Hecht *et al.*, Detection of perchlorate and the soluble chemistry of martian soil at the Phoenix lander site. *Science* **325**, 64–67 (2009). pmid: [19574385](https://pubmed.ncbi.nlm.nih.gov/19574385/)
26. J. M. Keller *et al.*, Equatorial and midlatitude distribution of chlorine measured by Mars Odyssey GRS. *J. Geophys. Res.* **111**, E03S08 (2006). doi: [10.1029/2006je002679](https://doi.org/10.1029/2006je002679)
27. D. C. Catling *et al.*, Atmospheric origins of perchlorate on Mars and the Atacama. *J. Geophys. Res.* **115**, E00E11 (2010). doi: [10.1029/2009JE003425](https://doi.org/10.1029/2009JE003425)
28. Y. S. Kim, K. P. Wo, S. Maity, S. K. Atreya, R. I. Kaiser, Radiation-induced formation of chlorine oxides and their potential role in the origin of Martian perchlorates. *J. Am. Chem. Soc.* **135**, 4910–4913 (2013). doi: [10.1021/ja3122922](https://doi.org/10.1021/ja3122922); pmid: [23506371](https://pubmed.ncbi.nlm.nih.gov/23506371/)
29. J. D. Schuttlefield, J. B. Sambur, M. Gelwicks, C. M. Eggleston, B. A. Parkinson, Photooxidation of chloride by oxide minerals: Implications for perchlorate on Mars. *J. Am. Chem. Soc.* **133**, 17521–17523 (2011). doi: [10.1021/ja2064878](https://doi.org/10.1021/ja2064878); pmid: [21961793](https://pubmed.ncbi.nlm.nih.gov/21961793/)
30. C. R. Webster *et al.*, Isotope ratios of H, C, and O in CO₂ and H₂O of the martian atmosphere. *Science* **341**, 260–263 (2013). doi: [10.1126/science.1237961](https://doi.org/10.1126/science.1237961); pmid: [23869013](https://pubmed.ncbi.nlm.nih.gov/23869013/)
31. R. E. Novak, M. J. Mumma, G. L. Villanueva, Measurement of the isotopic signatures of water on Mars; Implications for studying methane. *Planet. Space Sci.* **59**, 163–168 (2011). doi: [10.1016/j.pss.2010.06.017](https://doi.org/10.1016/j.pss.2010.06.017)
32. L. A. Leshin, S. Epstein, E. M. Stolper, Hydrogen isotope geochemistry of SNC meteorites. *Geochim. Cosmochim. Acta* **60**, 2635–2650 (1996). doi: [10.1016/0016-7037\(96\)00122-6](https://doi.org/10.1016/0016-7037(96)00122-6)
33. L. A. Leshin, Insights into martian water reservoirs from analyses of martian meteorite QUE94201. *Geophys. Res. Lett.* **27**, 2017–2020 (2000). doi: [10.1029/1999GL008455](https://doi.org/10.1029/1999GL008455)
34. P. R. Mahaffy *et al.*, Abundance and isotopic composition of gases in the martian atmosphere from the Curiosity rover. *Science* **341**, 263–266 (2013). doi: [10.1126/science.1237966](https://doi.org/10.1126/science.1237966); pmid: [23869014](https://pubmed.ncbi.nlm.nih.gov/23869014/)
35. I. P. Wright, M. M. Grady, C. T. Pillinger, Chassigny and the nakhlites: Carbon-bearing components and their relationship to martian environmental conditions. *Geochim. Cosmochim. Acta* **56**, 817–826 (1992). doi: [10.1016/0016-7037\(92\)90100-W](https://doi.org/10.1016/0016-7037(92)90100-W)
36. P. B. Niles, W. V. Boynton, J. H. Hoffman, D. W. Ming, D. Hamara, Stable isotope measurements of martian atmospheric CO₂ at the Phoenix landing site. *Science* **329**, 1334–1337 (2010). doi: [10.1126/science.1192863](https://doi.org/10.1126/science.1192863); pmid: [20829484](https://pubmed.ncbi.nlm.nih.gov/20829484/)
37. J. L. Bandfield, T. D. Glotch, P. R. Christensen, Spectroscopic identification of carbonate minerals in the martian dust. *Science* **301**, 1084–1087 (2003). doi: [10.1126/science.1088054](https://doi.org/10.1126/science.1088054); pmid: [12934004](https://pubmed.ncbi.nlm.nih.gov/12934004/)
38. J. Farquhar, J. Savarino, T. L. Jackson, M. H. Thiemens, Evidence of atmospheric sulphur in the martian regolith from sulphur isotopes in meteorites. *Nature* **404**, 50–52 (2000). doi: [10.1038/35003517](https://doi.org/10.1038/35003517); pmid: [10716436](https://pubmed.ncbi.nlm.nih.gov/10716436/)
39. J. P. Greenwood, L. R. Ricciuti, H. Y. McSweeney Jr., Sulfide isotopic compositions in shergottites and ALH84001, and possible implications for life on Mars. *Geochim. Cosmochim. Acta* **61**, 4449–4453 (1997). doi: [10.1016/S0016-7037\(97\)00246-9](https://doi.org/10.1016/S0016-7037(97)00246-9)
40. R. Navarro-González, E. Vargas, J. de la Rosa, A. C. Raga, C. P. McKay, Reanalysis of the Viking results suggests perchlorate and organics at midlatitudes on Mars. *J. Geophys. Res.* **115**, E12010 (2010). doi: [10.1029/2010JE003599](https://doi.org/10.1029/2010JE003599)
41. K. Biemann, J. L. Bada, Comment on “Reanalysis of the Viking results suggests perchlorate and organics at midlatitudes on Mars” by Rafael Navarro-González *et al.* *J. Geophys. Res.* **116**, E12001 (2011). doi: [10.1029/2011JE003869](https://doi.org/10.1029/2011JE003869)
42. S. C. Moldoveanu, *Analytical Pyrolysis of Natural Organic Polymers* (Elsevier, Amsterdam, 1998), pp. 496.
43. I. A. Franchi, I. P. Wright, A. S. Sexton, C. T. Pillinger, The oxygen-isotopic composition of Earth and Mars. *Meteorit. Planet. Sci.* **34**, 657–661 (1999). doi: [10.1111/j.1945-5100.1999.tb01371.x](https://doi.org/10.1111/j.1945-5100.1999.tb01371.x)
44. A. J. T. Jull, C. J. Eastoe, S. Clout, Isotopic composition of carbonates in the SNC meteorites, Allan Hills 84001 and Zagami. *J. Geophys. Res. Planets* **102**, 1663 (1997). doi: [10.1029/96JE03111](https://doi.org/10.1029/96JE03111)
45. P. B. Niles, L. A. Leshin, Y. Guan, Microscale carbon isotope variability in ALH84001 carbonates and a discussion of possible formation environments. *Geochim. Cosmochim. Acta* **69**, 2931–2944 (2005). doi: [10.1016/j.gca.2004.12.012](https://doi.org/10.1016/j.gca.2004.12.012)
46. I. P. Wright, R. H. Carr, C. T. Pillinger, Carbon abundance and isotopic studies of Shergotty and other shergottite meteorites. *Geochim. Cosmochim. Acta* **50**, 983–991 (1986). doi: [10.1016/0016-7037\(86\)90379-0](https://doi.org/10.1016/0016-7037(86)90379-0)
47. J. Hoefs, *Stable Isotope Geochemistry* (Springer-Verlag, Heidelberg, Germany, ed. 2, 1980).
48. M. A. Sephton, Organic compounds in carbonaceous meteorites. *Nat. Prod. Rep.* **19**, 292–311 (2002). doi: [10.1039/b103775g](https://doi.org/10.1039/b103775g); pmid: [12137279](https://pubmed.ncbi.nlm.nih.gov/12137279/)

Acknowledgments: NASA provided support for the development and operation of SAM, and for the SAM Science Team, led out of NASA's Goddard Space Flight Center. The GC-TCD subsystem for SAM was developed in France, with the support of CNES. The TLS subsystem for SAM was developed at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA. Data from the SAM experiment are archived in the Planetary Data System (pds.nasa.gov). The SAM development, operations, and testbed teams provided essential contributions to the successful operation of SAM on Mars and the acquisition of these data.

Supplementary Materials

www.sciencemag.org/content/341/6153/1238937/suppl/DC1
MSL Science Team Author List

9 April 2013; accepted 2 August 2013
[10.1126/science.1238937](https://doi.org/10.1126/science.1238937)

Electromagnetic Energy Conversion at Reconnection Fronts

V. Angelopoulos,^{1*} A. Runov,¹ X.-Z. Zhou,¹ D. L. Turner,¹ S. A. Kiehas,² S.-S. Li,¹ I. Shinohara³

Earth's magnetotail contains magnetic energy derived from the kinetic energy of the solar wind. Conversion of that energy back to particle energy ultimately powers Earth's auroras, heats the magnetospheric plasma, and energizes the Van Allen radiation belts. Where and how such electromagnetic energy conversion occurs has been unclear. Using a conjunction between eight spacecraft, we show that this conversion takes place within fronts of recently reconnected magnetic flux, predominantly at 1- to 10-electron inertial length scale, intense electrical current sheets (tens to hundreds of nanoamperes per square meter). Launched continually during intervals of geomagnetic activity, these reconnection outflow flux fronts convert ~10 to 100 gigawatts per square Earth radius of power, consistent with local magnetic flux transport, and a few times 10^{15} joules of magnetic energy, consistent with global magnetotail flux reduction.

Magnetic reconnection is a fundamental process of electromagnetic energy conversion into kinetic energy and heat that operates in the solar corona, astrophysical accretion disks, active galactic nuclei, and planetary magnetospheres. In Earth's space environment, reconnection facilitates solar wind energy input into the magnetosphere by enabling solar wind field lines to cross the magnetopause and be directly connected to Earth's field lines (Fig. 1A), stretching and compressing them into the magnetotail lobes. Increases in magnetotail lobe flux are evidence of the resultant magnetic energy storage. Nightside reconnection (X in Fig. 1A) converts this stored energy into particle motion, heating, or waves, moving it from the magnetosphere into the ionosphere or back to interplanetary space. Although energy storage and release often occur in distinct, easily recognizable cycles called substorms (1–4), transient magnetotail reconnection is, in fact, ubiquitous across all types of geomagnetic activity (5, 6).

After a pulse of transient reconnection in the magnetotail, magnetic flux transport and energy conversion are expected along the entire path of the flux bundle as it shrinks earthward or tailward from the reconnection point (red arrows in Fig. 1A) propelled by its curvature force (7). This electromagnetic energy conversion's nature and agreement with global substorm-scale energy estimates have been elusive, however. Energy conversion equals $\int J_y E_y dt dx dy dz$, where J_y is the dominant tail current; E_y is the dominant electric field (both duskward); and x , y , and z are Geocentric Solar Magnetospheric (GSM) coordinates (8). When evaluating the power conversion rate, $\mathbf{J} \cdot \mathbf{E}$, electron-scale processes must be distinguished from ion-scale pro-

cesses. This can be achieved by comparing $\mathbf{J} \cdot \mathbf{E}$ with $\mathbf{J} \cdot \mathbf{E}_{\text{MHD}}$, where $\mathbf{E}$ is the measured electric field and $\mathbf{E}_{\text{MHD}}$ is obtained from ion flow measurements and the frozen-in condition (8), consistent with

ideal and Hall magnetohydrodynamics (MHD). This requires comprehensive particles and fields instrumentation and high-time resolution measurements. In the past, there has been a lack of multipoint, high-time resolution, comprehensive plasma observations that can assess the nature, intensity, spatial extent, and duration of the most effective conversion and transport, concurrently with a global monitor of the amount of energy storage and release in the magnetosphere. Here, we use a fortuitous conjunction between the dual, lunar-orbiting mission ARTEMIS (Acceleration, Reconnection, Turbulence, and Electrodynamics of Moon's Interaction with the Sun) and six Earth-orbiting satellites in the magnetotail to measure local and global energy conversion and determine the location and properties of the most effective electromagnetic energy conversion process.

Substorm Observations

Because magnetic energy conversion results in plasma flows, heating, and waves and is related to magnetic flux transport, our event selection was motivated by the availability of spacecraft on

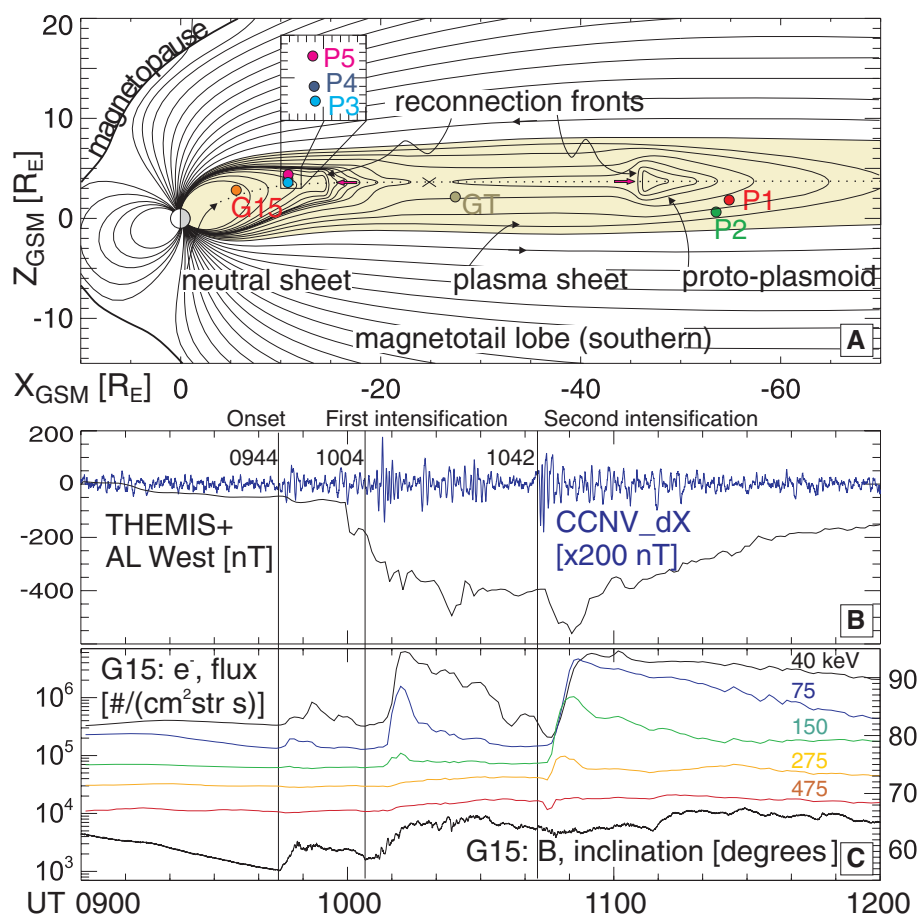


Fig. 1. Magnetotail configuration and geomagnetic activity analysis. (A) X-Z satellite projections and a sketch of the magnetotail configuration on 3 July 2012, 09:40 UT, obtained by modifying the T96 model field (32). GSM coordinates are used throughout this paper unless noted; X, Y, Z point roughly sunward, duskward, and northward, respectively (8). (B) High-pass-filtered (<120-s) magnetic pulsation data from Carson City, NV, and auroral electrojet index from Western THEMIS and ancillary stations. (C) Electron differential flux and B-field inclination at G15.

¹Department of Earth, Planetary and Space Sciences and Institute of Geophysics and Planetary Physics, University of California Los Angeles, 595 Charles Young Drive East, Los Angeles, CA 90095–1567, USA. ²Space Research Institute, Austrian Academy of Sciences, Schmiedlstrasse 6, 8042, Graz, Austria. ³Institute of Space and Astronautical Science, JAXA, 3-1-1 Yoshinodai, Chuo, Sagami-hara, Kanagawa 252-5210, Japan.

*Corresponding author. E-mail: vassilis@ucla.edu

both sides (earthward and tailward) of magnetic reconnection, where such phenomena have been observed previously. The ARTEMIS lunar orbiters P1 and P2 traverse Earth's magnetotail once per lunar month (9, 10). Once per year, when the three highly eccentric Earth orbiters THEMIS (Time History of Events and Macroscale Interactions during Substorms) P3, P4, and P5 reach their apogee (geocentric radius of apogee, $r_a \sim 12 R_E$) at premidnight, concurrent reconnection outflow flux measurements at both sides of a reconnection site can be made. During such a conjunction on 3 July 2012 at 9 to 12 UT, a substorm occurred.

At that time, Geotail (GT, $r_a \sim 30 R_E$) (11) and GOES (Geostationary Operational Environmental Satellites) 13 and 15 (G13 and G15, at $r_a \sim 6.6 R_E$) were also well positioned in the nightside magnetosphere (Fig. 1A and fig. S1).

Ground magnetic pulsations (Fig. 1B) and abrupt changes in the auroral electrojet low (AL) index enable detailed timing (8) of substorm onset and its two intensifications, demarcated by vertical lines in Fig. 1B. This timing is corroborated by radiation belt electron injections and magnetic field dipolarizations at G15 (Fig. 1C). The activity was centered at premidnight, exactly

where THEMIS and ARTEMIS were situated. The negative interplanetary field B_z throughout this time resulted in substantial magnetotail flux loading (fig. S3). The satellite locations, the interplanetary conditions, and the activity indices suggest that the observation conditions during this interval were appropriate for detection of magnetic energy conversion. But where should these energy conversion sites be expected to reside within these observations?

Earthward-moving reconnected flux bundles are typically associated with 10-min time-scale bursty bulk flows (BBFs) that envelop individual 1-min time-scale flow bursts and have a nominal $10 R_E^2$ Y-Z cross-section (12, 13). Even though BBFs are the most efficient transporters of flux and energy in the magnetotail, integrated BBF transport is small compared with total transport during substorms [see table 5 in (13) and table 1 and figure C1 in (14)], perhaps because spatial aliasing prohibits detection of full BBF duration or extent (13). Even if assumed continuous (and thus integrated) over substorm time scales (1.5 hours), BBF transport is smaller than substorm-scale transport of magnetic flux (0.5 to 1 GWb) by a factor of 10 and of energy (1×10^{15} to 5×10^{15} J) by a factor of 100. For the largest events (14), flux transport appears sufficient, but energy transport is still deficient by a factor of 3. Loads of considerable magnitude ($\sim 25 \text{ pW/m}^3$) lasting 1 to 10 min, possibly related to BBFs, have been reported (15, 16). Based on 4-s resolution time-delay analysis from the Cluster mission, such loads have been considered mostly stationary. When integrated over time and over their scale estimate (3 to $8 R_E$) along the X direction (15), their maximum power is 0.9×10^{13} to $2.5 \times 10^{13} \text{ J/R}_E^2$, smaller by a factor of 100 than expected per R_E^2 in cross-sectional area during a substorm.

However, recently appreciated intense current densities embedded within flow bursts (10 to 100 nA/m^2) could be potential sites of substantial electromagnetic power conversion (17). Defined by their rapid positive B_z excursion, these dipolarization fronts propagate over tens of R_E from the reconnection region to the inner magnetosphere. Correlated with negative density gradients of ion inertial length scale-size, intense waves, and plasma heating, these fronts indeed have the hallmarks of power conversion sites (18). Simulations of reconnection show that ion dynamics at such fronts are uniquely capable of substantial electromechanical power conversion (19). Although kinetic simulations (19, 20) differ on the exact location of the dominant conversion region, they agree that electron-scale power conversion becomes important only near the X point. Such simulations, however, are not global. Various boundary conditions could explain differences between simulations as well as potential differences between simulations and data.

The front's saddle-shaped current sheet (21, 22), which is at least partly supplied by the equatorial (XY) cross-tail current, can feed into the global substorm field-aligned current system at high latitudes,

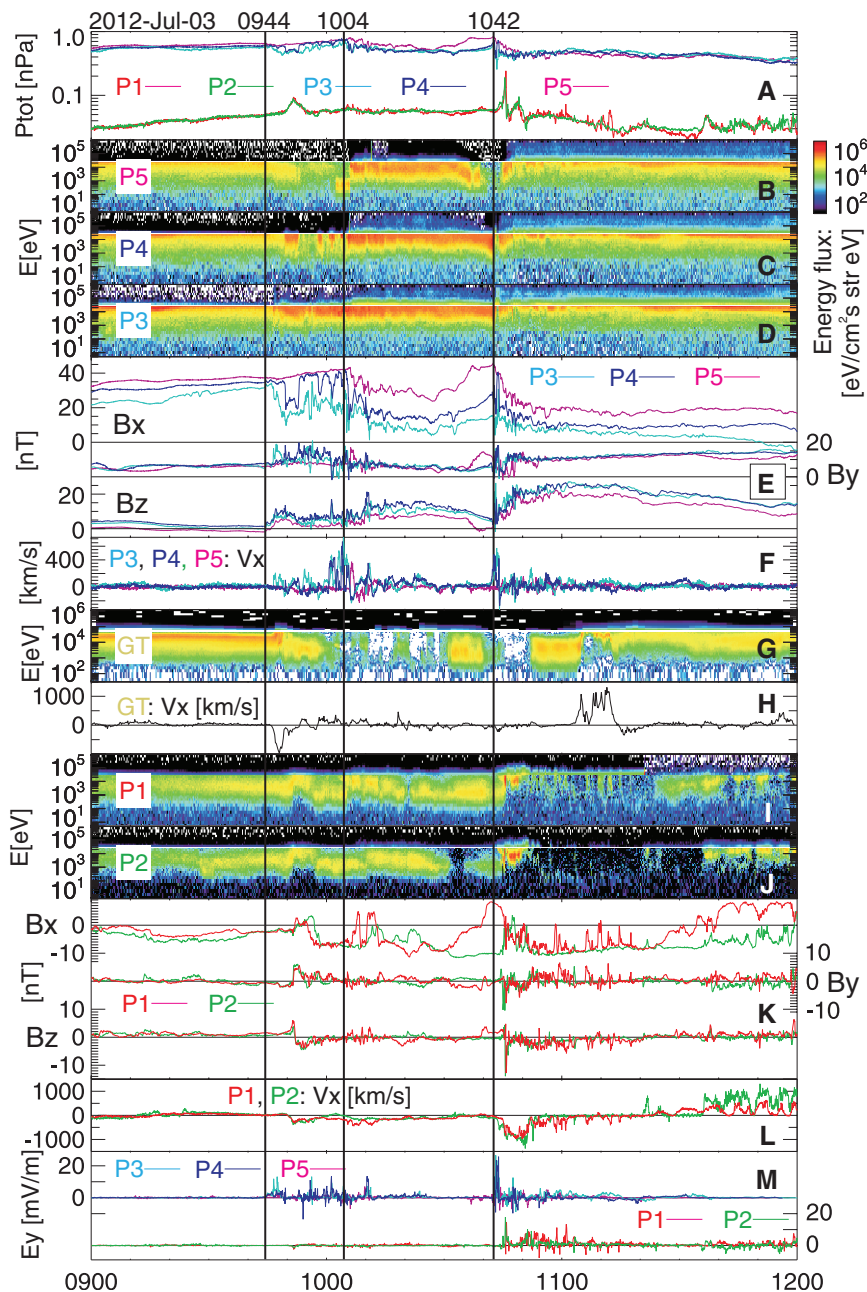


Fig. 2. Magnetotail data overview. (A) Total (magnetic plus plasma) pressure. (B, C, D, G, I, and J) Energy flux spectrograms. (E and K) Magnetic field on THEMIS and ARTEMIS (Geotail's field was unavailable due to Earth shadow). (F, H, and L) Ion X-component flows. (M) Measured Y-component electric field. Vertical lines are transferred from Fig. 1B.

with global consequences. Similar to its parent, non-BBF equatorial cross-tail current (23), the ion inertial length scale front current is often consistent with the Hall term in Ohm's law (17), except when the density gradient within it becomes so steep that electron pressure gradients and electron inertia start to dominate (22). Establishing the locations, longevity, and global evolution of these fronts through our multispacecraft observations could potentially result in adequate levels of integrated power conversion and transport.

The same pitfalls in assessing global transport and energy conversion also apply on the tailward side of the reconnection site, where reconnected flux bundles ram into ambient tail plasma, forming proto-plasmoids (Fig. 1A). These evolve into large-scale plasmoids (24) and are eventually released into the solar wind. Negative B_z excursions associated with tailward flows and density depletions also observed in the magnetotail were interpreted as putative counterparts of dipolarization fronts on the tailward side of the reconnection region (25). However, the relationship between such tailward reconnection outflow flux fronts and plasmoid formation and expulsion is unclear. Moreover, the presence, nature, and similarity of physical processes (flux transport, current formation, and energy conversion) at reconnection outflow flux fronts (or "reconnection fronts") on the two sides of a reconnection point have yet to be evaluated. Our multipoint measurements from the event selected address these questions and establish the longevity of power conversion sites within tailward-moving plasmas for comparison with global power conversion and flux transport estimates.

At ARTEMIS, total pressure peaks (Fig. 2A, at ~09:52 and ~10:46 UT) accompanied by bipolar B_z signatures and tailward flows (Fig. 2, K to L) (also see expanded views in figs. S5, K to L, and S6, K to L) indicate plasmoid passage, which is evidence of near-Earth reconnection. The plasmoids were followed by multiple bipolar B_z variations indicative of smaller plasmoids at P1 at 10:04 to 10:14 UT (after the first intensification) and at both P1 and P2 at 10:46 to 11:20 UT (after the second intensification), suggesting multiple instances of reconnection following each intensification. Integrating in situ flux transport measurements around plasmoid traversals is a poor measure of global flux transport because the spacecraft often enter the tail lobe after plasmoid passage. The low density, increased Debye length, and increased spacecraft potential magnitude at the lobe preclude in situ flux transport measurements there, consistent with our expectation of spatial aliasing in evaluating tailward transport. ARTEMIS's location in the distant tail, however, enables previously unavailable measurements of global flux transport, and its conversion rate and duration, for accurate comparisons with locally measured transport.

Lobe Flux: Storage and Conversion

Using P2's total pressure inside the magnetopause and the solar wind dynamic pressure outside it allowed us (8) to determine and integrate

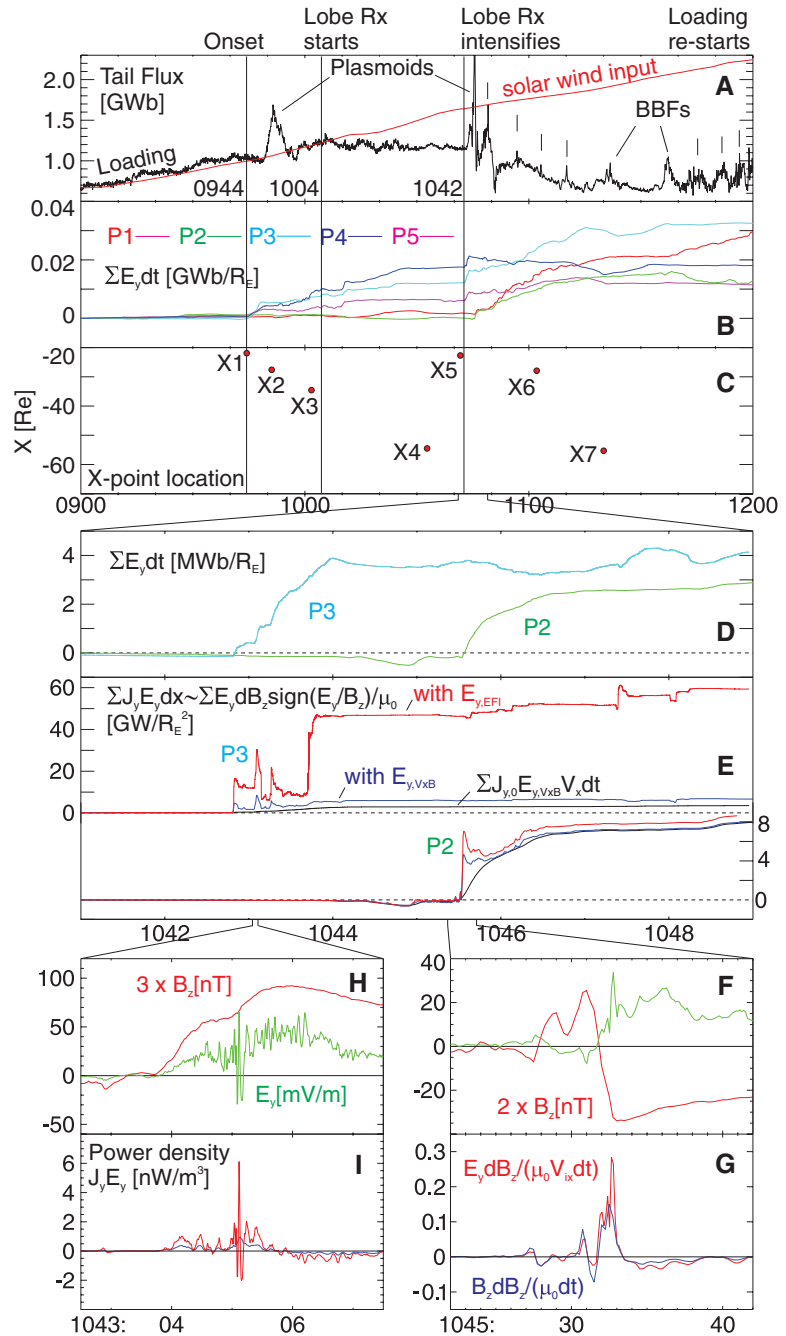


Fig. 3. Magnetotail flux transport and power conversion. (A) Solar wind magnetotail flux loading (red) (fig. S3E) and instantaneous magnetotail lobe flux, Φ (black) (figs. S7 and S8). (B) Cumulative flux transport past ARTEMIS and THEMIS, per unit of Y distance, estimated from the measured electric field. (C) Location and timing of reconnection impulses derived from time delays of waves and particles measured at P1 to P5 and GT as tabulated in table S1 [also see (8) and figs. S9 to S13]. Reconnection sites progress from near-Earth to ARTEMIS in two cycles; during each cycle, the sites remain earthward of 60 R_E . (D and E) Eight-minute detail of flux transport per unit of Y distance and energy conversion per unit Y -Z area observed past P2 and P3 after the second intensification. Dashed lines are zero levels. Red and blue in (E) are the cumulative power conversion due to the measured electric field or the MHD approximation. Black is the nominal cross-tail current, 0.5 nA/m², times the MHD electric field, for comparison with the contribution from reconnection fronts. Note the different scales for P3 (left) and P2 (right). (F) Detail (25-s) of E_y , B_z during front passage by P2. (G) Tailward-moving power conversion density $J_y E_y$ at P2 in the MHD approximation (blue) and using the measured E_y (red); (also see fig. S15 for context). (H and I) Five-second detail of the same quantities at P3, as in (F) and (G), for P2 (also see fig. S16 for context). Durations in (F) to (I) were chosen to enable sufficient resolution of the electron-scale structures.

the (presumed) monotonically decreasing magnetopause flaring angle, α , to obtain the tail radius, R_T , at P2. We used a variant of a technique that has been validated against simulations and observations (26). During our event, R_T varied between 35 and 38 R_E (8).

Next, using R_T and the magnetotail lobe field inferred from the total pressure at P2, we obtained the lobe flux, Φ , and compared it with the solar wind input (Fig. 3A). The sharp Φ increases and decreases demarcated by short vertical lines in the panel are not global; they correspond to plasmoid or BBF passage at P2, when intense local curvature or inertial forces invalidate our implicit approximation of a planar, quasistatic plasma sheet. If they are ignored, Φ agrees well with the solar wind input before the first substorm intensification, supporting our finding that lobe reconnection did not accompany the first plasmoid expulsion. For half an hour after the first intensification, however, substantial reduction in lobe flux relative to the solar wind input was observed, suggesting that lobe reconnection sufficient to balance the solar wind input was taking place.

The most notable lobe flux reduction during the three-hour interval of interest occurred within 30 min after the second substorm intensification (10:42 UT), when Φ decreased ~ 0.5 GWb (by a factor of two). By 11:35 UT, Φ began to increase again, signifying a new cycle of lobe magnetic flux and energy loading from the solar wind. Cumulative flux transport past P1 to P5 (Fig. 3B), which occurred in short-lived bursts, differed considerably at the five satellites. We attribute this to spatial aliasing, as activity moves away from the spacecraft, or as the spacecraft moves into the lobes where particles and electric field are barely measurable. Because magnetic flux is conserved, however, the increasing difference between solar wind input and Φ (Fig. 3A) is proof of ongoing magnetotail transport; the recurrence of plasmoids and BBFs suggests that such transport occurs continually over substorm time scales (several tens of minutes).

Reconnection Site Locations

During a substorm, magnetic reconnection in the magnetotail is not stationary but has been observed to progress downtail in a series of impulsive, localized activations (27). In our event, this motion needs to be understood to permit integration of $\mathbf{J} \cdot \mathbf{E}$ over a volume that contains the X point. Reconnection locations can be determined by timing emitted MHD wave pulses (28), energy dispersion of accelerated particles (29, 30), or flow reversals. By using such standard methods (8), we found (Fig. 3C and table S1) that reconnection started near $X \sim -23 R_E$ at substorm onset and progressed tailward of ARTEMIS by 10:32 UT. Thereafter, the current sheet thinned globally, as evidenced by the increased B_x difference between P1 and P2 and between P5 and P4 (Fig. 2, E and K). Subsequently, a new reconnection site formed near Earth (X5, $X \sim -23 R_E$) and initiated a new sequence of tailward-moving

activations (X6 and X7). Thus, in the ~ 30 min after the second intensification, the reconnection site remained earthward of ARTEMIS, suggesting that a reasonable approximation of the magnetotail at that time is a cylinder bounded by $X = 0$ and $-60 R_E$, where energy flows out along $\pm X$ only from the high-beta plasma sheet, mostly as particle energy recently converted from magnetic energy. Because reconnection remained in this cylinder, all magnetic energy conversion occurred inside it.

Reconnection Fronts

Within the plasmoids observed here and elsewhere at a similar distance (24), tailward reconnection fronts usher low-density, hotter, presumably recently reconnected plasma (Fig. 2, I to L) (also see greater detail in figs. S5, I to L, and S6, I to L). These structures may be proto-plasmoids, formed by interaction of newly reconnected flux bundles with ambient plasma, as observed recently in simulations (31). The sharp interface between ambient and recently reconnected plasma (e.g., 10:45 UT) contains an intense westward cross-tail current. Using the 12-s time delay of the B_z minimum from P2 to P1 produces a speed of ~ 600 km/s, consistent with the in situ measured ion velocity, 400 to 800 km/s (Fig. 2H and figs. S11H and S12H). The similarity of the front at P1 and P2 also indicates that it lasts much longer than the ~ 2 -s observation period at each satellite; its speed suggests that it emanated from the X5 reconnection site (Fig. 3C and table S1) several minutes earlier.

The observed 20 nT B_z decrease over 2-s results in estimates of 13 nA/m² and 1200 km for the front's average current density and thickness (~ 2 to 3 ion inertial lengths; ~ 1 to 2 ion gyroradii). The order-of-magnitude density drop; ion heating; and intense E_y (~ 13 mV/m), which deviates very little from the MHD approximation near the peak $|B_z|$ and just behind the front; and an E_x at the front consistent with a Hall current (fig. S14, A to G) are also classical dipolarization front signatures (17), except that they have a reverse B_z . Again, as for dipolarization fronts, this tailward reconnection front's speed, estimated from multiple spacecraft observations, agrees fairly well with the ion flow, V_x , validating our approximation $dx \sim -V_x dt$ for converting temporal to spatial derivatives, at least near the front.

The main terms in $\mathbf{J} \cdot \mathbf{E}$ during front motion are $J_y E_y \sim (-\partial B_z / \partial x) E_y / \mu_0 + J_{\alpha} E_y$, where $J_{\alpha} = (\partial B_x / \partial z) / \mu_0$ is the equatorial cross-tail non-BBF current (~ 0.5 nA/m²), which is at least 10 times smaller than the front current. Because $dx \sim -V_x dt$, $J_y \sim (\partial B_z / \partial t) / V_x \mu_0$ provides a reasonable first-order estimate of the dominant current. In MHD, $E_y \sim V_x B_z$ and $\mathbf{J} \cdot \mathbf{E}_{\text{MHD}} = (\partial B_z / \partial t) B_z / \mu_0$. This approximation holds even when the Hall term is substantial, as we see by replacing V_x with V_{xe} in J_y and in E_y . When electron terms in Ohm's law dominate, the Hall electric field in the X direction no longer equals the measured electric field in the plasma frame: $J_y B_z / Ne \neq E_x + (\mathbf{V} \times \mathbf{B})_x$, and power conversion $J_y E_y \sim (\partial B_z / \partial t) E_y / V_x \mu_0$

disagrees with its MHD approximation. Such situations are interesting and important because they can lead to energy conversion in the electron rest frame at the fronts.

During the tailward reconnection front's passage past P1, the electromagnetic power conversion density $J_y E_y \sim (\partial B_z / \partial t) E_y / V_x \mu_0$ agrees well with its MHD equivalent $(\partial B_z / \partial t) B_z / \mu_0$, as expected when electrons are frozen into the field (fig. S14H). This agreement is also consistent with the expectation of a Hall current at dipolarization fronts of an ion inertial length scale gradient (17). These observations support the notions that physical processes at reconnection fronts on both sides of a reconnection site are identical and that on the tailward side, such fronts participate in early plasmoid formation. As we shall see below, these reconnection front similarities on both sides of the reconnection site extend to processes at electron scales.

Energy Conversion at Electron Inertial Length Scales

Density and field gradients of scale size shorter than an ion inertial length can develop at dipolarization fronts; electron pressure gradients and electron inertia are then needed to support these currents (22). Tailward reconnection fronts are apparently no exception: Despite gross similarities in particle spectra and magnetic field at P1 and P2, tailward reconnection front behavior at P2 (Fig. 3, F and G, and fig. S15) differs markedly from that at P1. At P2, the spin-fit E_y differs from $(\mathbf{V} \times \mathbf{B})_y$ by $\sim 30\%$ (fig. S15F). The high-resolution E_y (8 samples/s) peaks at >30 mV/m over 250-msec (~ 150 km, or 4 electron inertial lengths given the density of 0.02 cm^{-3}) when the density and magnetic field gradients within the front are steepest (Fig. 3F and fig. S15, C and F). At that time, the Hall electric field $J_y B_z / Ne$ differs dramatically from the measured E_x in the plasma frame (fig. S15G), implying that electron terms in Ohm's law are important. At the same time, the power conversion density $J_y E_y \sim (\partial B_z / \partial t) E_y / V_x \mu_0$ (Fig. 3G and fig. S15H) is a factor of 2 larger ($>250 \text{ pW/m}^3$) than if electrons were frozen into the plasma (Fig. 3G and fig. S15H) and twice the maximum value reported previously (15, 16).

At P3, several reconnection fronts (Fig. 2E and figs. S6E and S16C) that exhibit classical dipolarization front signatures are embedded within the BBF counterpart to the second plasmoid (Fig. 2F). Yet the spin-fit E_y can deviate by a factor of 2 from $(\mathbf{V} \times \mathbf{B})_y$ (fig. S16E), and at high-time resolution (128 samples/s) it exhibits peaks >50 mV/m (Fig. 3H and fig. S16F) at the sharpest gradients. During those times, $E_x + (\mathbf{V} \times \mathbf{B})_x \neq J_y B_z / Ne$ (fig. S16H), implying that electron terms in Ohm's law are important. The earthward-moving power conversion density $J_y E_y \sim (\partial B_z / \partial t) E_y / V_x \mu_0$ is an order of magnitude larger than its MHD approximation for about 250-msec. It exhibits peaks $>6000 \text{ pW/m}^2$ over time scales of 50-msec (20 km given the ~ 400 km/s speed of these structures, or ~ 1 to 2 electron inertial lengths given the density of 0.2 cm^{-3}). The observed power conversion

density is two orders of magnitude larger than previously reported (15, 16).

The power conversion density at the fronts drives sizeable cumulative increases in power conversion past the satellites (Fig. 3E). Because the difference between the integral of $J_y E_y$ and its MHD approximation (the difference between the red and blue curves) is large, the cumulative power conversion is, in great part, due to the nonideal terms in Ohm's law. Thus, electron inertial length scale-size substructures at reconnection fronts are efficient power conversion regions. Contrary to our expectation from past simulations (19, 20), such regions are not limited to the vicinity of the X point. Could such small-scale, locally transient, power conversion rates match the expected global energy conversion during this typical substorm?

Global Consequences

Integrating the electromagnetic energy conservation equation (9) over a cylindrical magnetotail volume capped at $X = 0$ and $-60 R_E$, and assuming negligible tangential magnetic flux transport into the volume at the magnetopause boundary (slow solar wind flux loading) and out of the volume at the two caps of the cylinder (at the high-beta plasma sheet), we expect the magnetic energy reduction in that volume to equal the time and space integral of the magnetic power conversion within it. Because of the spatially coherent propagation of reconnection fronts across large distances, known on the earthward side (17) and observed here on the tailward side (Fig. 2K and fig. S6K), we expect power conversion at the fronts to continue over substorm time scales, not just for the short times observed in situ. The reconnection fronts that followed the second intensification converted 5 to 50 GW/ R_E^2 of power past P2 and P3 (Fig. 3E and figs. S15K and S16K). This occurred during the major local flux transport (3 to 4 MWb/ R_E) (Fig. 3D and figs. S15, J and L, and S16, J and L).

Because most tail flux reduction during the substorm took place over the ensuing 30-min period, we examined whether power conversion at these fronts is consistent with the magnetic energy reduction in the aforementioned integration volume associated with local flux transport and global flux reduction over the same time period.

The magnetic energy W_L within a portion of the near-Earth tail length $L \sim 60 R_E$ is $W_L = L^2 \Phi^2 / 2\mu_0 A$; it varies during a substorm because both area (A) and flux (Φ) vary: $dW_L/W_L = 2d\Phi/\Phi - dA/A$. In our case, the tail radius was reduced from 38 R_E to 35 R_E (fig. S8C), and Φ from 1.2 to 0.7 GWb (Fig. 3A and fig. S8C); thus, W_L decreased from 4.8×10^{15} to 1.9×10^{15} J, releasing $\Delta W_L \sim 2.9 \times 10^{15}$ J of magnetic energy. This global energy conversion needs to be compared with the integrated power conversion from plasma sheet measurements. Because $\Delta A/A$ was smaller by a factor of 2.7 than $\Delta\Phi/\Phi$, we can approximate $\Delta W_L/W_L \sim 1.64(\Delta\Phi/\Phi)$.

First, we consider energy conversion related to local flux transport. The measured flux transport per unit of Y distance past P3 ($X_{P3} = -11 R_E$)

was $\Delta\Phi/\Delta Y = \Sigma E_y dt \sim 4$ MWb/ R_E (Fig. 3D). This flux arrived from the X5 reconnection site at $X_5 = -23 R_E$, as a $\Delta X = X_{P3} - X_5 = 12 R_E$ -long lobe flux bundle reconnected and contracted. The expected energy conversion per unit of Y distance integrated earthward of X5 is $(\Delta W_{\Delta X}/\Delta Y)/W_{\Delta X} = (\Delta W_L/W_L)/\Delta Y = 1.64(\Delta\Phi/\Phi)/\Delta Y \sim 1.64(4 \text{ MWb}/R_E)/(1.2 \text{ GWb}) \sim 5.5 \times 10^{-3}/R_E$, where $W_{\Delta X} = (\Delta X/L)W_L$ is the magnetic energy estimate of the lobe volume earthward of X5. Thus, the anticipated energy conversion per unit of Y distance based on local flux measurements is $\Delta W_{\Delta X}/\Delta Y \sim 5.5 \times 10^{-3} W_{\Delta X}/R_E \sim 5.5 \times 10^{-3} (\Delta X/L)W_L/R_E \sim 5.5 \times 10^{-3} (12/60) 4.8 \times 10^{15} \text{ J}/R_E$ or $\Delta W_{\Delta X}/\Delta Y \sim 5.3 \times 10^{12} \text{ J}/R_E$. This has a $\pm 10\%$ uncertainty due to the location of the X5 site (8) (fig. S13) but considerably larger uncertainty due to the simplicity of the cylindrical tail model used.

The observed cumulative spatial (along X) integral of the power conversion density accomplished by the reconnection front flux bundles transported past P3 was 50 GW/ R_E^2 (Fig. 3E). Assuming that the reconnected flux bundles started to shrink earthward at 10:41:35 UT (exact timing from fig. S13) and continued for 145 s until the flows ceased locally at 10:44:00 UT (Figs. 3D and 2F and fig. S6F), and that the curved flux bundle is $\Delta Z \sim 3 R_E$ tall in the Z direction, this shrinkage resulted in $50 \text{ GW}/R_E^2 \times 3 R_E \times 145 \text{ s} \sim 22 \times 10^{12} \text{ J}/R_E$ energy release per unit of Y distance. This is several times larger than expected from $\Delta W_{\Delta X}/\Delta Y$ above. Thus, even if reconnection fronts converted energy at the measured rate for a fraction of their lifetime, they could still account for the entire energy conversion associated with the locally observed flux transport in the reconnection outflow region.

Second, we consider energy conversion related to global flux transport. Although flow bursts are localized in Y (1 to 3 R_E) (12), their effective interaction width ΔY_{eff} can be larger due to a multiplicity of bursts along X or Y , multiple fronts within each flow burst, or aliasing from flow burst stoppage or rebound at the spacecraft location. This effective interaction width can be determined from flux conservation. The average flux transport per unit time we observed is (4 MWb/ R_E /145-s), and the total transport must be 0.5 GWb/1800-s. Assuming that transport proceeds continuously during that time, $\Delta Y_{\text{eff}} \sim (0.5 \text{ GWb}/1800\text{-s})/(4 \text{ MWb}/R_E/145\text{-s}) \sim 10 R_E$. The energy conversion in the same region is therefore $50 \text{ GW}/R_E^2 \times \Delta Y_{\text{eff}} \times \Delta Z \times 1800\text{-s} \sim 2.7 \times 10^{15} \text{ J}$. On the tailward side, at P2, the observed power conversion is smaller by a factor of 10 (5 GW/ R_E^2); all else being roughly equal, this adds $\sim 0.3 \times 10^{15} \text{ J}$ to the total energy conversion, resulting in a total of $3 \times 10^{15} \text{ J}$, close to the aforementioned expected energy conversion from lobe flux reduction, $\Delta W_L \sim 2.9 \times 10^{15} \text{ J}$, during this typical substorm. Thus, the integrated power conversion at the fronts is also consistent with global magnetic energy conversion during this event.

From both in situ measured magnetic flux transport in the outflow region and global magnetic flux reduction, we conclude that electron

inertial length scale processes at reconnection fronts play a key role in magnetic energy conversion during magnetic reconnection.

References and Notes

1. S.-I. Akasofu, *Physics of Magnetospheric Substorms* (D. Reidel, Norwell, MA, 1977).
2. R. L. McPherron, *Rev. Geophys.* **17**, 657–681 (1979).
3. D. N. Baker, T. I. Pulkkinen, V. Angelopoulos, W. Baumjohann, R. L. McPherron, *J. Geophys. Res.* **101**, 12975–13010 (1996).
4. W. I. Axford, *Phys. Chem. Earth C* **24**, 147–151 (1999).
5. V. A. Sergeev, R. J. Pellinen, T. I. Pulkkinen, *Space Sci. Rev.* **75**, 551–604 (1996).
6. Y. Kamide et al., *J. Geophys. Res.* **103**, 17705–17728 (1998).
7. S. S. Li et al., *J. Geophys. Res.* **116**, A00135 (2011).
8. Materials and methods are available as supplementary materials on Science Online.
9. V. Angelopoulos, *Space Sci. Rev.* **165**, 3–25 (2011).
10. V. Angelopoulos, *Space Sci. Rev.* **141**, 5–34 (2008).
11. A. Nishida, *Geophys. Res. Lett.* **21**, 2871–2873 (1994).
12. R. Nakamura et al., *Geophys. Res. Lett.* **31**, L09804 (2004).
13. V. Angelopoulos et al., *J. Geophys. Res.* **99**, 21257 (1994).
14. V. Angelopoulos et al., *J. Geophys. Res.* **102**, 211 (1997).
15. M. Hamrin et al., *J. Geophys. Res.* **116**, A00K08 (2011).
16. O. Marghita et al., in *The Cluster Active Archive*, H. Laakso, M. Taylor, C. P. Escoubet, Eds. (Springer, New York, 2010), pp. 453–459; doi:10.1007/978-90-481-3499-132.
17. A. Runov et al., *J. Geophys. Res.* **116**, A05216 (2011).
18. V. A. Sergeev et al., *Geophys. Res. Lett.* **36**, L21105 (2009).
19. M. I. Sitnov, M. Swisdak, A. V. Divin, *J. Geophys. Res.* **114**, A04202 (2009).
20. J. Birn, M. Hesse, *Ann. Geophys.* **23**, 3365–3373 (2005).
21. J. Liu, V. Angelopoulos, A. Runov, X.-Z. Zhou, *J. Geophys. Res.* **118**, 2000–2020 (2013).
22. X.-J. Zhang et al., *J. Geophys. Res.* **116**, A00120 (2011).
23. V. Angelopoulos et al., *Geophys. Res. Lett.* **20**, 1711–1714 (1993).
24. S. S. Li, V. Angelopoulos, A. Runov, S. A. Kiehas, X.-Z. Zhou, *J. Geophys. Res.* **118**, 2133–2144 (2013).
25. S.-I. Ohtani, M. A. Shay, T. Mukai, *J. Geophys. Res.* **109**, A03210 (2004).
26. M. A. Shukhtina, E. I. Gordeev, V. A. Sergeev, *Ann. Geophys.* **27**, 1583–1591 (2009).
27. V. Angelopoulos et al., *J. Geophys. Res.* **101**, 24599 (1996).
28. J. Liu et al., *J. Geophys. Res.* **116**, A00117 (2011).
29. E. T. Sarris et al., *J. Geomag. Geoelectr.* **48**, 649–656 (1996).
30. I. G. Richardson, S. W. H. Cowley, *J. Geophys. Res.* **90**, 12133 (1985).
31. M. Ugai, *Ann. Geophys.* **29**, 1411–1422 (2011).
32. N. A. Tsyganenko, *J. Geophys. Res.* **100**, 5599 (1995).

Acknowledgments: Funded by NASA NAS5-02099 and Austrian Science Fund (FWF) J3041-N16. Data obtained from: <http://themis.ssl.berkeley.edu>. We thank J. Hohl and E. Masongsong for editorial help, the THEMIS and ancillary data providers (<http://themis.igpp.ucla.edu/roadrules.shtml>), National Oceanic and Atmospheric Administration for the GOES data, NASA/SPDF for the solar wind data (http://omniweb.gsfc.nasa.gov/ow_min.html), and WDD-C2 for the AL index.

Supplementary Materials

www.sciencemag.org/content/341/6153/1478/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S16
Table S1
References (33–39)

25 February 2013; accepted 22 August 2013
10.1126/science.1236992

Circadian Gene *Bmal1* Regulates Diurnal Oscillations of Ly6C^{hi} Inflammatory Monocytes

Khoa D. Nguyen,^{1,2} Sarah J. Fentress,³ Yifu Qiu,¹ Karen Yun,¹ Jeffery S. Cox,³ Ajay Chawla^{1,4*}

Circadian clocks have evolved to regulate physiologic and behavioral rhythms in anticipation of changes in the environment. Although the molecular clock is present in innate immune cells, its role in monocyte homeostasis remains unknown. Here, we report that Ly6C^{hi} inflammatory monocytes exhibit diurnal variation, which controls their trafficking to sites of inflammation. This cyclic pattern of trafficking confers protection against *Listeria monocytogenes* and is regulated by the repressive activity of the circadian gene *Bmal1*. Accordingly, myeloid cell-specific deletion of *Bmal1* induces expression of monocyte-attracting chemokines and disrupts rhythmic cycling of Ly6C^{hi} monocytes, predisposing mice to development of pathologies associated with acute and chronic inflammation. These findings have unveiled a critical role for BMAL1 in controlling the diurnal rhythms in Ly6C^{hi} monocyte numbers.

The circadian clock is a timekeeping system that allows organisms to adapt their physiological and behavioral rhythms to anticipatory changes in their environment (1, 2). In mammals, the circadian timing system has a hierarchical architecture, consisting of the light-

responsive central clock in the suprachiasmatic nuclei and the peripheral clocks that are present in virtually all cells of the body (3). Whereas the central clock entrains and synchronizes the peripheral clocks with the day-night cycle, the peripheral clocks regulate tissue-specific programs in an anticipatory manner. Although the peripheral clock has been identified in macrophages (4–9), its role in anticipatory immune responses remains poorly understood.

In simple terms, the inflammatory response can be expressed as a product of inducible gene expression in an innate cell multiplied by the number of infiltrating innate cells. When examined from this viewpoint, circadian oscil-

lations could potentially regulate inflammatory responses by modulating rhythmic expression of inflammatory genes in tissue macrophages or by controlling rhythmic trafficking of Ly6C^{hi} inflammatory monocytes (10, 11). Because the cumulative cost incurred by rhythmic expression of inflammatory genes is likely to be high (in terms of tissue inflammation and damage), we postulated that rhythmic mobilization of Ly6C^{hi} monocytes provides a better means of mounting anticipatory inflammatory responses. In this scenario, the rhythmic mobilization of Ly6C^{hi} monocytes would fortify the host's innate immune defenses in anticipation of environmental challenges, a process we term anticipatory inflammation.

Results

Diurnal Oscillations and Trafficking of Ly6C^{hi} Monocytes

To investigate this hypothesis, we examined whether blood monocytes exhibited diurnal variation in expression of clock genes. An analysis of monocytes obtained from mice kept under a 12-hour light-dark cycle revealed rhythmic expression of messenger RNA (mRNA) encoded by the clock gene *Bmal1* (*Arntl*), whose oscillation was antiphasic to its two target genes, *Nr1d1* and *Dbp* (Fig. 1A). A similar rhythm was observed for luciferase protein in monocytes derived from *Per2^{Luc}* knock-in mice (12), which express PERIOD2 as a luciferase fusion protein (fig S1A). Furthermore, serum synchronization of THP-1 cells, a human monocytic cell line, revealed a 24-hour oscillation of clock genes

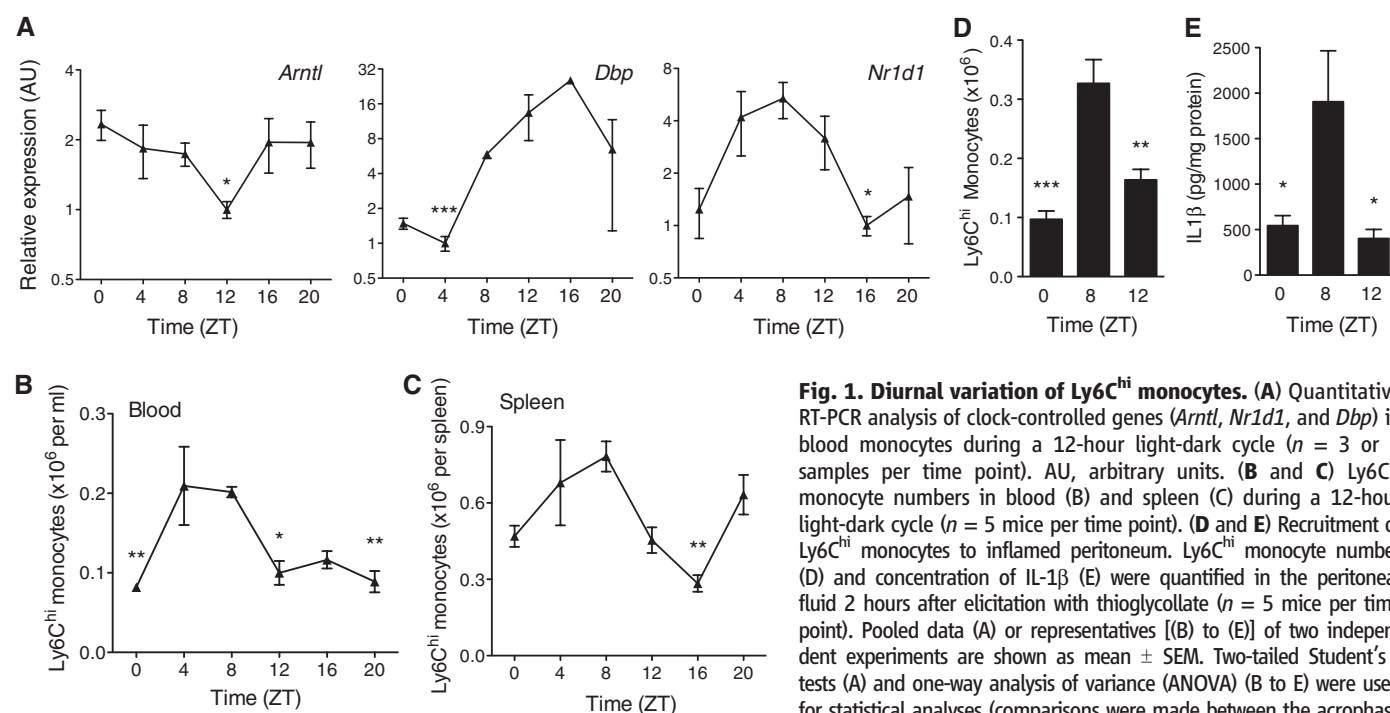


Fig. 1. Diurnal variation of Ly6C^{hi} monocytes. (A) Quantitative RT-PCR analysis of clock-controlled genes (*Arntl*, *Nr1d1*, and *Dbp*) in blood monocytes during a 12-hour light-dark cycle ($n = 3$ or 4 samples per time point). AU, arbitrary units. (B and C) Ly6C^{hi} monocyte numbers in blood (B) and spleen (C) during a 12-hour light-dark cycle ($n = 5$ mice per time point). (D and E) Recruitment of Ly6C^{hi} monocytes to inflamed peritoneum. Ly6C^{hi} monocyte number (D) and concentration of IL-1 β (E) were quantified in the peritoneal fluid 2 hours after elicitation with thioglycollate ($n = 5$ mice per time point). Pooled data (A) or representatives [(B) to (E)] of two independent experiments are shown as mean $\pm$ SEM. Two-tailed Student's t tests (A) and one-way analysis of variance (ANOVA) (B to E) were used for statistical analyses (comparisons were made between the acrophase and other time points). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

under constant culture conditions (fig. S1B). Together, these data demonstrate that blood monocytes exhibit diurnal variation in clock genes, which might impart rhythmicity to their functions.

Recent studies have demonstrated rhythmic trafficking of immune cells into tissues (6, 8), prompting us to ask whether monocyte frequency in the three major reservoirs (blood, spleen, and bone marrow) also varies in a diurnal manner. Indeed, between the peak and nadir Zeitgeber time (ZT, where ZT0 refers to lights on and ZT12 refers to lights off), there was ~twofold difference in the total number of monocytes present in blood and spleen (figs. S2 and S3, A and B), with bone marrow displaying a reciprocal diurnal rhythm (fig. S3C).

We next examined whether the observed oscillations in total monocytes resulted from rhythmic changes in Ly6C^{hi} (inflammatory) or Ly6C^{low} (patrolling) monocytes (13, 14). The peak and nadir of Ly6C^{hi} monocytes in blood and spleen mirrored the cyclic pattern of total monocytes in these reservoirs (Fig. 1, B and C, and fig. S3, A and B), whereas Ly6C^{low} monocytes did not display strong oscillatory behavior in blood or spleen (fig. S3, D and E). Moreover, the expression of *Ccr2* mRNA in monocytes did not correlate with Ly6C^{hi} monocyte numbers in

blood (fig. S3F). Because Ly6C^{hi} monocytes are recruited to sites of inflammation (15, 16), we investigated the relation between diurnal variations in Ly6C^{hi} monocyte numbers and monocyte-driven inflammation by using the thioglycollate model of sterile peritonitis (17). The numbers of Ly6C^{hi} monocytes recruited to the inflamed peritoneum at ZT8 were ~threefold higher than at ZT0 (Fig. 1D), which resulted in ~3 to 3.5-fold higher inflammation, as quantified by the release of interleukin (IL)-1 β and IL-6 (Fig. 1E and fig. S3G). On a per cell basis, expression of IL-1 and IL-6 did not exhibit diurnal oscillations (fig. S3, H and I), suggesting that diurnal variation in Ly6C^{hi} monocyte numbers dictates the magnitude of the innate inflammatory response. Moreover, monocyte-attracting chemokines CCL2 and CCL8, whose concentrations were higher (~two- to threefold) in the inflamed peritoneum at ZT8 (fig. S3, J and K), were primarily secreted by recruited monocytes and resident macrophages but not neutrophils (fig. S3L).

Diurnal Rhythms of Ly6C^{hi} Monocytes During *Listeria monocytogenes* Infection

Ly6C^{hi} monocytes provide the first-line defense against *L. monocytogenes* (18), leading us to posit that anticipatory oscillations in Ly6C^{hi} monocyte numbers might modulate innate responses

to infection. To investigate this hypothesis, we intraperitoneally infected C57BL/6J mice with *L. monocytogenes* at ZT0 or ZT8. Two days post-infection (dpi), the peritoneum, spleen, and liver of mice infected at ZT8 had significantly fewer bacteria than those infected at ZT0 (Fig. 2, A to C). Improved bacterial clearance at ZT8 was associated with ~2.2- to 3.8-fold higher numbers of TNF (tumor necrosis factor)/iNOS (inducible nitric oxide synthase)-producing (Tip)-dendritic cells (DCs), which differentiate from Ly6C^{hi} monocytes to control the growth of this pathogen (Fig. 2, D to F, and fig. S4) (19). Moreover, serum and, to an even greater extent, peritoneum concentrations of chemokines and cytokines necessary for the mobilization and activation of Ly6C^{hi} monocytes were much higher at ZT8 than at ZT0 (fig. S5A and Fig. 2G). This was associated with higher recruitment of Ly6C^{hi} monocytes but not neutrophils to the peritoneum (fig. S5, B and C), findings that are consistent with previous observations that neutrophils are dispensable for defense against *L. monocytogenes* (20).

Although Ly6C^{hi} monocytes and Tip-DCs limit bacterial growth during the early phase of infection, efficient clearance of *L. monocytogenes* requires adaptive immunity (18), prompting us to investigate the activation of adaptive immunity 6 dpi. Although the peritoneal cavity was

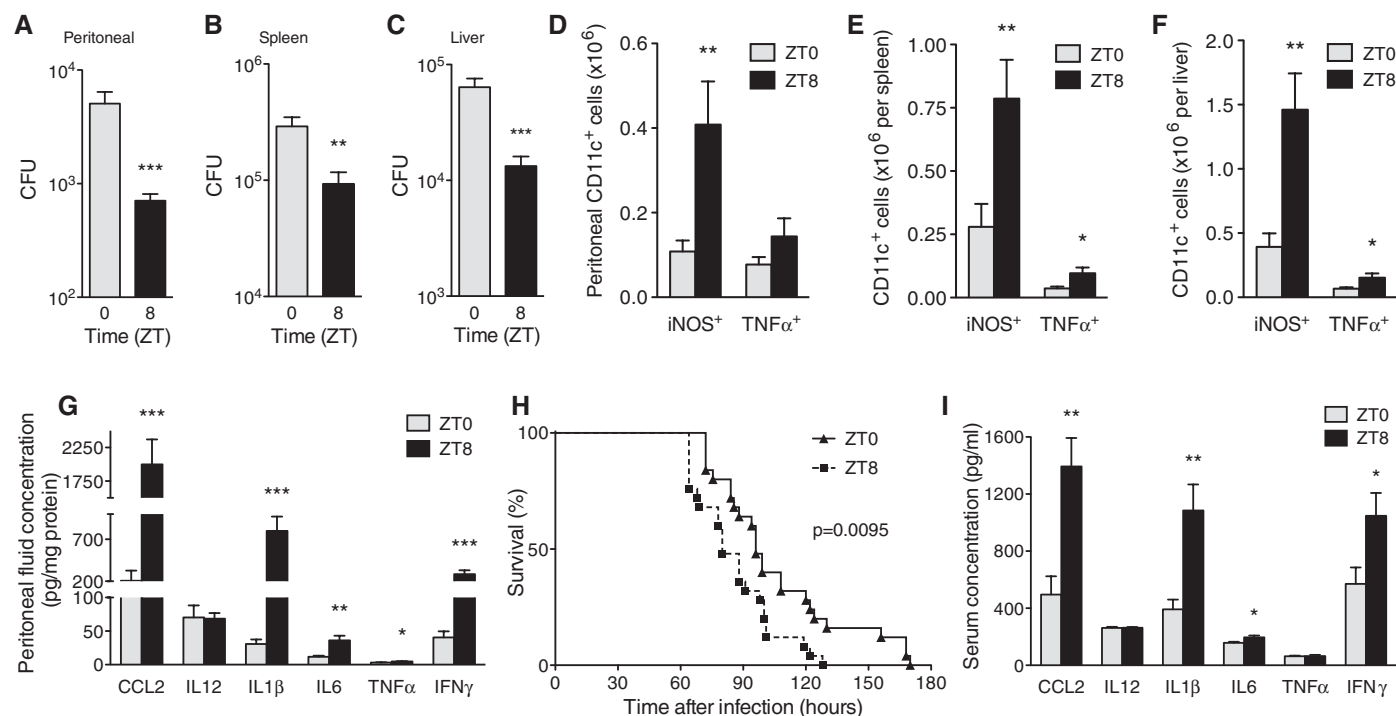


Fig. 2. Diurnal variation in the pathogenicity of *L. monocytogenes*. (A to C) Mice kept under a 12-hour light-dark cycle were intraperitoneally inoculated with 1×10^6 *L. monocytogenes* at ZT0 and ZT8, and CFUs recovered from the peritoneal cavity (A), spleen (B), and liver (C) were quantified 2 dpi ($n = 14$ or 15 mice per time point). (D to F) Numbers of $\text{iNOS}^+\text{CD11c}^+$ and $\text{TNF}\alpha^+\text{CD11c}^+$ cells in peritoneal cavity (D), spleen (E), and liver (F) of mice 2 dpi with 1×10^6 *L. monocytogenes* at ZT0 and ZT8 ($n = 15$ mice per time point). (G) Concentration of chemokines and cyto-

kines in peritoneal fluid 2 dpi with 1×10^6 *L. monocytogenes* at ZT0 and ZT8 ($n = 15$ mice per time point). (H) Survival curves of mice after infection with 1×10^7 *L. monocytogenes* at ZT0 and ZT8 ($n = 25$ mice per time point). (I) Serum concentration of chemokines and cytokines 2 dpi with 1×10^7 *L. monocytogenes* at ZT0 and ZT8 ($n = 10$ mice per time point). Pooled data from two or three independent experiments are presented as mean $\pm$ SEM. Statistical significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) was assessed by using two-tailed Student's *t*-test [(A) to (G) and (I)] and log-rank test (H).

below the limit of detection, mice inoculated at ZT8 continued to demonstrate enhanced clearance of *L. monocytogenes* in secondary sites, such as the liver (fig. S5D). In contrast, bacterial burden in the spleen was not significantly different between mice inoculated at ZT0 and ZT8, especially after normalization for spleen size (fig. S5, E to G). Infection at ZT8 resulted in stronger adaptive immune response in the spleen and liver, as evidenced by ~3- to 4.5-fold higher numbers of interferon- γ (IFN- γ)-producing CD4⁺ and CD8⁺ T cells at ZT8 (figs. S4 and S5, H and I). Tip-DCs were also more numerous in spleen and liver, but not the peritoneum, at 6 dpi (fig. S5, J to L).

Infection with a high inoculum of pathogens often results in overactivation of the immune system, causing systemic inflammation and death (11). Because previous studies have demonstrated circadian influence on sepsis-induced mortality (8, 21, 22), we investigated whether the host response to infection with a higher dose of *L. monocytogenes* might exhibit similar diurnal variation. Intraperitoneal infection of mice with 1×10^7 *L. monocytogenes* resulted in significantly higher mortality rate at ZT8 than at ZT0 (Fig. 2H). This increase in mortality was not associated with a higher bacterial burden (fig. S6, A to C) but rather with an enhanced inflammatory response (Fig. 2I). These results demonstrate that the host response to *L. monocytogenes* ex-

hibits diurnal rhythms that parallel the rhythms of Ly6C^{hi} monocytes.

BMAL1 Regulates Rhythmic Oscillations of Ly6C^{hi} Monocytes

To determine whether clock genes in monocytes regulate their diurnal rhythms, we generated myeloid-specific *Bmal1* knockout mice using *Arntl*^{LoxP/LoxP} and *Lyz2*^{Cre} mice (designated *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre}) (23). Immunoblot analysis confirmed loss of BMAL1 protein in blood monocytes of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice (fig. S7A). Quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of mRNAs provided further verification that diurnal variations of core clock genes, including *Arntl* and *Nr1d1*, were abolished in blood monocytes of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice (fig. S7B and Fig. 3A). Remarkably, the disruption of BMAL1 expression in myeloid cells was sufficient to impair the diurnal variations in Ly6C^{hi} monocyte numbers in blood, spleen, and bone marrow (Fig. 3, B to D). The normal diurnal rhythm of total monocytes was similarly disrupted in the blood and spleens of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice (fig. S7, C and D). In contrast, we failed to detect rhythmic changes in the numbers of Ly6C^{low} monocytes (fig. S7, E and F) and neutrophils (fig. S7, G and H) in control (*Arntl*^{LoxP/LoxP}) and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice. These results demon-

strate that BMAL1 regulates the rhythmic oscillations of Ly6C^{hi} monocyte numbers in all three monocyte reservoirs.

We next tested whether disruption of the diurnal rhythms of Ly6C^{hi} monocytes alters their trafficking patterns. Unlike the diurnal recruitment of Ly6C^{hi} monocytes in *Arntl*^{LoxP/LoxP} mice, the inflamed peritoneum of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice had higher numbers of Ly6C^{hi} monocytes, which lacked rhythmicity (fig. S8A). These changes were specific for Ly6C^{hi} monocytes because recruitment of total monocytes did not exhibit a diurnal pattern (fig. S8B). Moreover, there were no significant differences between the genotypes in the numbers of total monocytes, Ly6C^{hi} monocytes, neutrophils, or macrophages in the uninflamed peritoneum (fig. S8, C to F). However, the increased recruitment of Ly6C^{hi} monocytes did amplify the local inflammatory response in *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice, as quantified by the release of CCL2, CCL8, IL-1 β , and IL-6 (fig. S9, A to D). This increase in peritoneal inflammation was again independent of diurnal changes in the expression of *Il1b* and *Il6* (fig. S9, E to F).

The amplification of inflammation in *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice suggested that these animals might be predisposed to developing infection-induced systemic inflammation. To test this hypothesis, we infected *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice at ZT0 and ZT8 with a

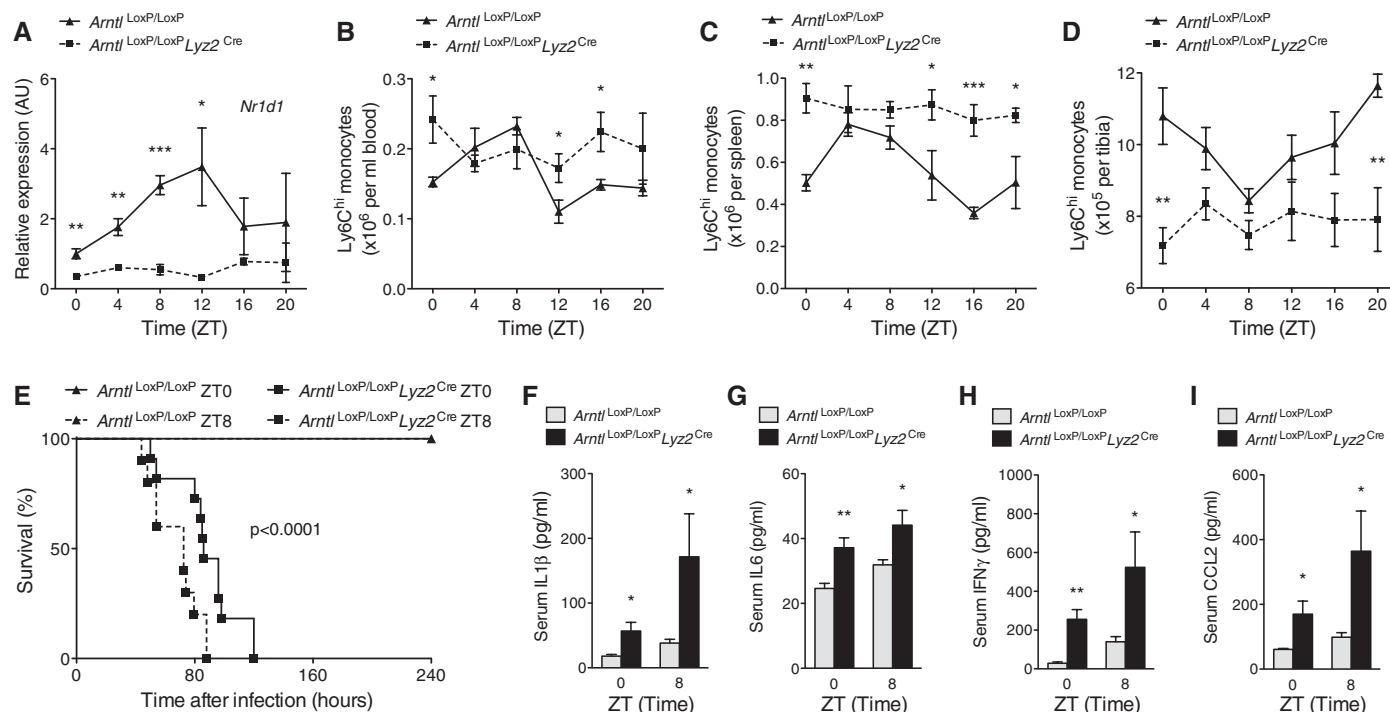


Fig. 3. BMAL1 regulates rhythmic oscillations of Ly6C^{hi} monocytes in a cell-autonomous manner. (A) Quantitative RT-PCR kinetic analysis of *Nr1d1* mRNA in blood monocytes isolated from *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice kept on a 12-hour light-dark cycle ($n = 3$ or 4 samples per genotype and time point). (B to D) Ly6C^{hi} monocyte numbers in blood (B), spleen (C), and bone marrow (D) of *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice kept under a 12-hour light-dark cycle at various ZTs ($n = 5$ mice per genotype and time point). (E) Survival curves of *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice

after infection with 1×10^6 *L. monocytogenes* at ZT0 and ZT8 ($n = 10$ or 11 mice per genotype and time point). (F to I) Serum concentrations of IL-1 β (F), IL-6 (G), IFN- γ (H), and CCL2 (I) in *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice 2 dpi with 1×10^6 *L. monocytogenes* at ZT0 and ZT8 ($n = 4$ to 6 mice per genotype and time point). Pooled data [(A) and (E)] and representative [(B) to (D)] of two to three independent experiments are shown as mean $\pm$ SEM and analyzed by using two-tailed Student's *t* tests [(A) to (D)] and log-rank test (E). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

nonlethal dose of *L. monocytogenes* and monitored their survival. Compared with *Arntl*^{LoxP/LoxP} mice, all *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice exhibited greatly reduced survival with median survival times of 77 to 91 hours (Fig. 3E), which could not be accounted for by differences in expression of *Thr2* or *Thr5* (fig. S9, G and H), two pattern recognition receptors that have been implicated in the recognition of *L. monocytogenes* (24, 25). However, *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice infected at ZT8 were slightly more susceptible to infection-induced lethality than those infected at ZT0 (Fig. 3E), perhaps reflecting incomplete depletion of BMAL1 protein in the newly recruited bone marrow monocytes (18).

An analysis of sera 2 dpi confirmed that *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice had higher circulating concentration of inflammatory cytokines and chemokines, including IL-1 β , IL-6, IFN- γ , and CCL2 (Fig. 3, F to I). This increase in systemic inflammation occurred in the absence of worsening infection because bacterial colony-forming units (CFUs) in the spleens and livers

of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice were lower or unchanged, respectively (fig. S10, A and B), whereas those in peritoneum were marginally higher (fig. S10C). Congruent with the CFU data, spleens rather than livers of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice exhibited a more robust increase in numbers of Tip-DCs and IFN- γ -producing CD4⁺ and CD8⁺ T cells (fig. S10, D to K). These data show that BMAL1-dependent diurnal rhythms of Ly6C^{hi} monocytes confers a survival advantage during an infectious challenge with *L. monocytogenes*.

BMAL1 Recruits PRC2 Complex to Repress Chemokine Genes

The recruitment of Ly6C^{hi} monocytes to inflammatory sites is mediated by the chemokine receptor CCR2 and its ligands, such as CCL2 and CCL8 (26). We observed that expression of CCL2, CCL8, and S100A8, a small calcium-binding protein implicated in monocyte chemotaxis (27), is regulated in a diurnal manner in monocytes recruited to sites of inflammation (fig. S11, A to C). These observations led to us to ask whether

BMAL1/CLOCK heterodimers might directly regulate chemokine gene expression in monocytes and macrophages. Indeed, deletion of *Arntl* resulted in higher expression of all three chemokine genes (*Ccl2*, *Ccl8*, and *S100a8*) in monocytes and peritoneal macrophages (Fig. 4A and fig. S12, A to E), which contributed to increased concentrations of CCL2 and CCL8 in the serum (fig. S12, F and G). These data suggest that repression by BMAL1 is necessary to generate diurnal rhythms in chemokine expression. Furthermore, bioinformatic analyses confirmed that promoter regions of *Ccl2*, *Ccl8*, and *S100a8* contained E-box motifs to which both BMAL1 and CLOCK were recruited in a rhythmic manner (Fig. 4B and fig. S13, A to E). This rhythmic recruitment of BMAL1 or CLOCK to the chemokine promoters was absent in bone marrow-derived macrophages (BMDMs) lacking BMAL1 (Fig. 4B and fig. S13, A to E), suggesting that BMAL1/CLOCK heterodimers might recruit a repressor complex to silence chemokine gene expression.

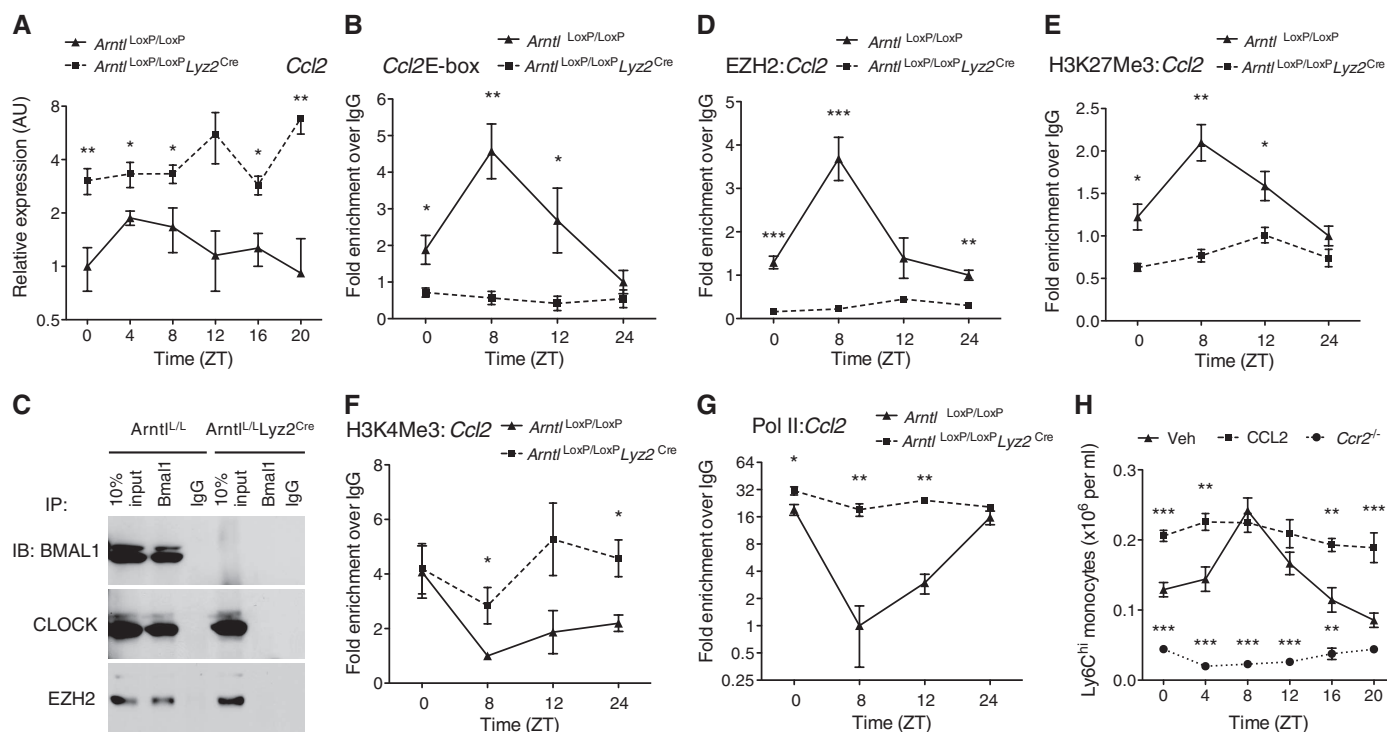


Fig. 4. BMAL1 recruits PRC2 to repress expression of *Ccl2*. (A) Quantitative RT-PCR analysis of *Ccl2* expression in blood monocytes of *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice kept on a 12-hour day-light cycle ($n = 3$ or 4 samples per genotype and time point). (B) ChIP analysis of BMAL1 binding to the *Ccl2* promoter ($n = 4$ samples per genotype and time point). (C) Coimmunoprecipitation of BMAL1 and with members of PRC2. Nuclear lysates from serum-shocked BMDMs were immunoprecipitated (IP) with BMAL1 antibody and immunoblotted (IB) for BMAL1, CLOCK, EZH2, EED, and SUZ12. Ig, immunoglobulin G. (D and G) ChIP analysis for the recruitment of EZH2 (D) and Pol II (G) to the proximal promoter of the *Ccl2* gene ($n = 4$ samples per genotype and time point). (E and F) ChIP analysis for H3K27Me3 (E) and H3K4Me3 (F) at the proximal promoter of *Ccl2* gene ($n = 4$ samples per genotype and time point). (H) Ly6C^{hi} monocyte numbers in the blood of wild-type or *Ccr2*^{-/-} mice during a 12-hour light-dark cycle. Wild-type mice were intraperitoneally injected with phosphate-buffered saline (Veh) or CCL2 (20 μ g kg⁻¹) 24 hours before quantification of Ly6C^{hi} monocytes ($n = 4$ or 5 mice per genotype/treatment and time point). Pooled data [(A) to (G)] from two independent experiments are shown as mean $\pm$ SEM and analyzed by using two-tailed Student's *t* tests [(A), (B), and (D) to (G)] and two-way ANOVA (H). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ represent comparison between *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} or between wild-type-treated Veh versus CCL2 or *Ccr2*^{-/-} at each time point.

Previous studies have demonstrated that histone acetylation and methylation is important in circadian gene expression (28, 29). Among the epigenetic marks that regulate clock-controlled genes (CCGs), trimethylation of histone H3 at Lys²⁷ (H3K27Me3) by polycomb repressive complex 2 (PRC2) has been implicated in the silencing of CCGs (30), prompting us to ask whether BMAL1 can interact with members of PRC2 in BMDMs. Immunoprecipitation of endogenous BMAL1 not only pulled down CLOCK but also members of PRC2, including the histone methyltransferase EZH2 (enhancer of zeste), EED (extra-sex comb), and SUZ12 (suppressor of zeste) (Fig. 4C). This interaction was specific because we failed to pull down CLOCK or members of PRC2 in BMAL1-deficient BMDMs (Fig. 4C). Chromatin immunoprecipitation (ChIP) experiments revealed that EZH2 was rhythmically recruited to the proximal promoter of *Ccl2* gene in a BMAL1-dependent manner (Fig. 4D), which temporally coincided with its silencing by H3K27Me3 (Fig. 4E). Moreover, in the absence of BMAL1, the chromatin state of the *Ccl2* gene was more active, as evidenced by the presence of H3K4Me3 activation marks and the constitutive recruitment of RNA polymerase II (Pol II) to the promoter (Fig. 4, F and G). Similar patterns of EZH2 and RNA Pol II recruitment and the associated chromatin modifications were observed on *Ccl8*

(fig. S14, A to D) and *S100a8* promoters (fig. S15, A to D).

We next tested the importance of the CCL2-CCR2 chemokine axis in the maintenance of Ly6C^{hi} diurnal rhythms. Consistent with published reports, the number of total and Ly6C^{hi} monocytes were lower in blood and spleens of *Ccr2*^{-/-} mice (Fig. 4H and fig. S16, A to C) (31). However, loss of CCR2 also abolished the diurnal variation of total and Ly6C^{hi} monocytes in the blood and spleen (Fig. 4H and fig. S16, A to C). Because bone marrow monocyte content is antiphasic to that of the periphery, *Ccr2*^{-/-} mice had a higher number of Ly6C^{hi} monocytes throughout the time course (fig. S16D). In contrast, administration of CCL2 to C57BL/6J mice was sufficient to disrupt the diurnal oscillations of Ly6C^{hi} and total monocytes in all three reservoirs (Fig. 4H and fig. S16, A to D). These data indicate that the rhythmic recruitment of the PRC2 complex by BMAL1/CLOCK heterodimers imparts diurnal variation to chemokine expression that is necessary to sustain Ly6C^{hi} monocyte rhythms.

Myeloid Cell BMAL1 Deficiency Worsens Metabolic Disease

Having established a physiological role for the diurnal rhythms of Ly6C^{hi} monocytes during acute infection, we investigated whether their dis-

ruption contributes to pathogenesis of chronic inflammatory diseases. Our initial studies focused on diet-induced obesity and insulin resistance, because low-grade chronic inflammation has been shown to modulate the expression of these disease phenotypes (32, 33). We thus fed *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice a high-fat diet (HFD) for 1 week and monitored the recruitment of Ly6C^{hi} monocytes to metabolic tissues. Compared with *Arntl*^{LoxP/LoxP} mice, short-term HFD feeding induced Ly6C^{hi} monocytosis and increased the Ly6C^{hi} macrophage content of epididymal white adipose tissue (eWAT) and brown adipose tissue (BAT) of *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice (fig. S17, A to C). Moreover, in *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice, the newly recruited Ly6C^{hi} eWAT and BAT macrophages expressed higher levels of monocyte-attracting chemokines (fig. S17, D to F), suggesting that disruption of the diurnal rhythms of monocytes might potentiate metabolic inflammation and disease.

To explore this hypothesis, we fed *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice a HFD and monitored the development of metabolic disease. Compared with *Arntl*^{LoxP/LoxP} mice, *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice gained ~30% more weight on HFD, which contributed to their higher total body adiposity and increased tissue weight (Fig. 5, A to C). This increase in weight gain likely resulted from a decrease in energy expenditure, as reflected in

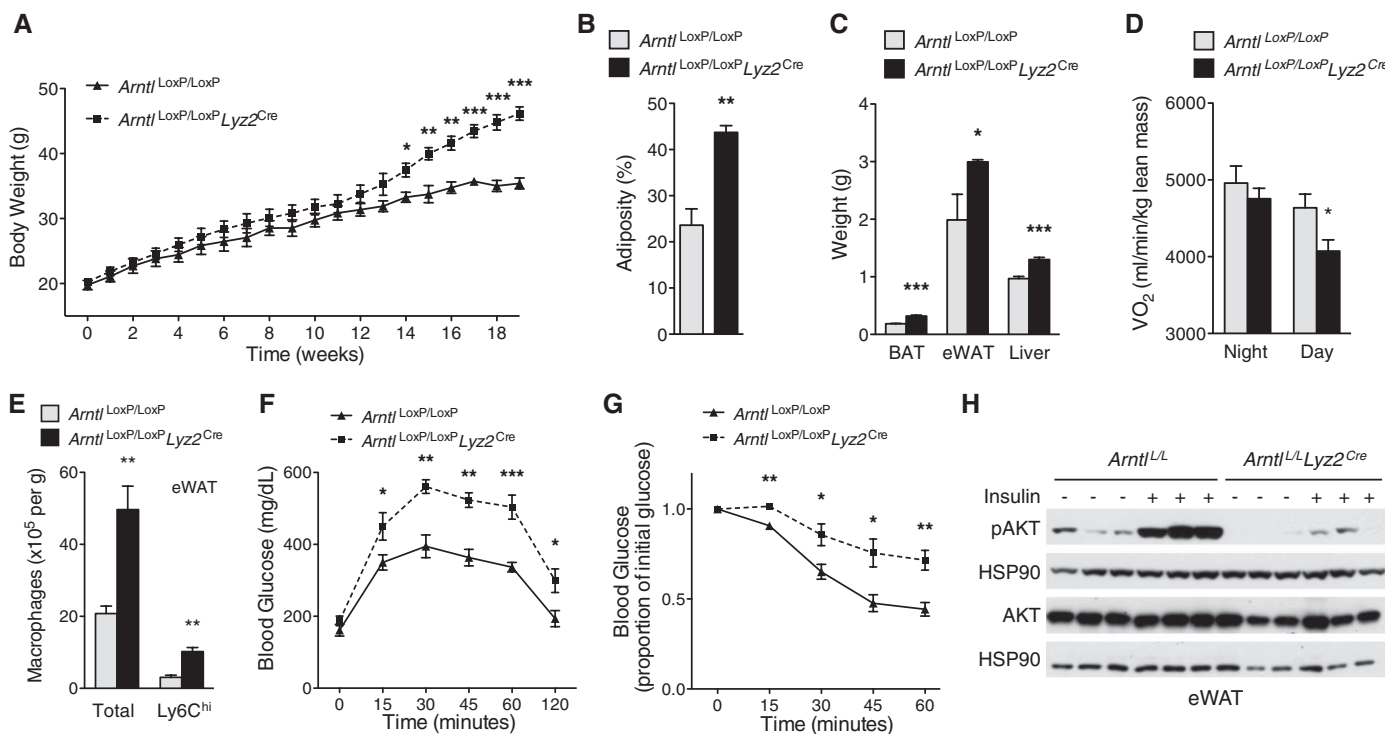


Fig. 5. Myeloid cell-specific deletion of BMAL1 exacerbates metabolic disease. (A to D) Body weight (A), adiposity (B), tissue weight (C), and oxygen consumption (D) in *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice kept on a 12-hour light-dark cycle fed a HFD for 19 weeks ($n = 4$ or 5 mice per genotype). (E) Total and Ly6C^{hi} macrophage content in eWAT of *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice fed a HFD ($n = 5$ mice per genotype). (F and G) Glucose (F) and insulin tolerance (G) tests of

Arntl^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} mice fed a HFD ($n = 5$ to 8 mice per genotype). (H) Immunoblots of total and phosphorylated Akt (pAKT) in eWAT of obese *Arntl*^{LoxP/LoxP} and *Arntl*^{LoxP/LoxP}*Lyz2*^{Cre} administered intraperitoneal insulin. HSP90, heat shock protein 90. Representative data of two to four independent experiments [(A) to (C) and (E) to (H)] are shown as mean $\pm$ SEM and analyzed by using two-tailed Student's *t* tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the lower oxygen consumption rate (~12%) of *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice during the day cycle (Fig. 5D). Food intake, total activity, and substrate utilization were not different between *Arntl^{LoxP/LoxP}* and *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice (fig. S18, A to C).

We next investigated whether chronic exposure to HFD exacerbated inflammation in metabolic tissues of *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice. Flow cytometric analysis revealed that *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice had higher numbers of total and Ly6C^{hi} (~2.5- and ~1.8-fold higher than *Arntl^{LoxP/LoxP}* mice, respectively) macrophages in their eWAT (Fig. 5E and fig. S19A). Macrophage subset analysis further showed that absolute numbers of CD11c⁺ and CD301⁺ macrophages were both higher in eWAT of *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice (fig. S19, A and B). These inflammatory changes were not restricted to eWAT because we observed similar increases in macrophage content of BAT in *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice (fig. S19, C and D). Congruent with these observations, there was evidence for increased local and systemic inflammation in *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice (fig. S20, A to C), including increased infiltration of eWAT and BAT by adaptive immune cells (fig. S20, D and E) and higher expression of monocyte-attracting chemokines (fig. S20, F to H). In contrast, body weight and macrophage content of eWAT and BAT were not significantly different in mice fed normal chow (fig. S21, A to C). These data demonstrate that deletion of *Arntl* in myeloid cells prevents diurnal oscillations between Ly6C^{hi} and Ly6C^{low} monocytes, which potentiates the inflammatory response to obesity.

On the basis of these findings, we assessed whether *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice were more prone to developing obesity-associated insulin resistance and metabolic disease. Glucose tolerance testing showed impaired clearance of glucose in *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice (Fig. 5F), which likely resulted from a decrease in systemic insulin action (Fig. 5G). Congruent with this postulate, insulin-induced serine phosphorylation of Akt was markedly impaired in eWAT, liver, and skeletal muscle of *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice (Fig. 5H and fig. S22, A and B). In addition, histological analysis demonstrated ectopic deposition of triglycerides in liver and BAT (fig. S22, C to F), and leukocytic infiltration in eWAT (fig. S22G), thus providing further evidence for worsening insulin resistance and metabolic dysfunction in *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice.

Because previous studies have demonstrated that changes in feeding period can entrain the peripheral cellular clocks (34), we lastly investigated whether monocyte diurnal rhythms are responsive to changes in nutrient intake. As expected, restricting feeding to the daytime induced a 12-hour phase shift in liver diurnal gene expression (fig. S23, A and B), whereas the expression of clock genes in peritoneal macrophages remained unchanged (fig. S23, C and D). Accordingly, the diurnal oscillations of Ly6C^{hi}

and total monocytes in all three reservoirs displayed similar rhythms irrespective of the feeding regimen (fig. S23, E to I), suggesting that Ly6C^{hi} monocyte rhythms primarily fortify host defenses against anticipatory changes in the environment.

Discussion

Previous studies have demonstrated bidirectional cross-talk between circadian clocks and metabolism (1, 2, 28). For instance, the peripheral clocks in metabolic tissues anticipate feeding-fasting cycles, and, conversely, feeding rhythms are strong Zeitgebers that can entrain peripheral clocks of metabolic tissues. In contrast, we found that the diurnal rhythms of myeloid cells do not anticipate metabolic rhythms and cannot be entrained by time-restricted feeding cycles. This suggests that the primary function of diurnal oscillations in myeloid cell numbers is not in anticipatory regulation of metabolism but perhaps in host defense. In support of this idea, both the time of infection and the myeloid-specific BMAL1 are critical determinants of the pathology associated with infectious challenge with *L. monocytogenes*. Moreover, the ability of CCL2, whose expression is induced upon sensing of pathogens, to override the diurnal oscillations in myeloid cell numbers provides a mechanism by which the host defenses can shift from anticipatory to pathogen-directed responses.

Although diurnal variation in monocyte numbers is not entrained by feeding cues, its disruption does render mice susceptible to diet-induced obesity and insulin resistance. The increased susceptibility of *Arntl^{LoxP/LoxP}Lyz2^{Cre}* mice to metabolic disease likely results from Ly6C^{hi} monocytosis and the subsequent recruitment of these cells into metabolically stressed adipose tissue. This potentiates the chronic inflammatory response both locally and systemically, resulting in insulin resistance and hyperglycemia. Last, because chronic inflammatory diseases, such as myocardial infarction, asthma, and rheumatoid arthritis, exhibit diurnal clustering in humans (35–37), rhythmic oscillations of inflammatory monocytes might also contribute to their pathogenesis.

References and Notes

- G. Asher, U. Schibler, *Cell Metab.* **13**, 125–137 (2011).
- J. Bass, J. S. Takahashi, *Science* **330**, 1349–1354 (2010).
- C. Saini, D. M. Suter, A. Liani, P. Gos, U. Schibler, *Cold Spring Harb. Symp. Quant. Biol.* **76**, 39–47 (2011).
- J. E. Gibbs *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 582–587 (2012).
- M. Keller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21407–21412 (2009).
- C. Scheiermann, Y. Kunisaki, P. S. Frenette, *Nat. Rev. Immunol.* **13**, 190–198 (2013).
- E. Haus, M. H. Smolensky, *Chronobiol. Int.* **16**, 581–622 (1999).
- C. Scheiermann *et al.*, *Immunity* **37**, 290–301 (2012).

- M. Hayashi, S. Shimba, M. Tezuka, *Biol. Pharm. Bull.* **30**, 621–626 (2007).
- F. Geissmann *et al.*, *Science* **327**, 656–661 (2010).
- R. Medzhitov, *Nature* **454**, 428–435 (2008).
- S. H. Yoo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5339–5346 (2004).
- C. Auffray *et al.*, *Science* **317**, 666–670 (2007).
- F. Geissmann, S. Jung, D. R. Littman, *Immunity* **19**, 71–82 (2003).
- L. Boring *et al.*, *J. Clin. Invest.* **100**, 2552–2561 (1997).
- C. S. Robbins, F. K. Swirski, *Cell. Mol. Life Sci.* **67**, 2685–2693 (2010).
- Materials and methods are available as supplementary materials on Science Online.
- N. V. Serbina, C. Shi, E. G. Pamer, *Adv. Immunol.* **113**, 119–134 (2012).
- N. V. Serbina, T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel, E. G. Pamer, *Immunity* **19**, 59–70 (2003).
- C. Shi *et al.*, *J. Immunol.* **187**, 5293–5298 (2011).
- A. C. Silver, A. Arjona, W. E. Walker, E. Fikrig, *Immunity* **36**, 251–261 (2012).
- P. G. Shackelford, R. D. Feigin, *Science* **182**, 285–287 (1973).
- K. A. Lamia, K. F. Storch, C. J. Weitz, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 15172–15177 (2008).
- F. Hayashi *et al.*, *Nature* **410**, 1099–1103 (2001).
- D. Torres *et al.*, *Infect. Immun.* **72**, 2131–2139 (2004).
- C. Shi, E. G. Pamer, *Nat. Rev. Immunol.* **11**, 762–774 (2011).
- C. J. Cornish *et al.*, *J. Cell. Physiol.* **166**, 427–437 (1996).
- D. Feng, M. A. Lazar, *Mol. Cell* **47**, 158–167 (2012).
- S. Sahar, P. Sassone-Corsi, *Handbook Exp. Pharmacol.* **217**, 29–44 (2013).
- J. P. Etchegaray *et al.*, *J. Biol. Chem.* **281**, 21209–21215 (2006).
- C. L. Tsou *et al.*, *J. Clin. Invest.* **117**, 902–909 (2007).
- G. S. Hotamisligil, *Nature* **444**, 860–867 (2006).
- J. I. Odegaard, A. Chawla, *Science* **339**, 172–177 (2013).
- F. Damiola *et al.*, *Genes Dev.* **14**, 2950–2961 (2000).
- R. J. Martin, *Am. Rev. Respir. Dis.* **147**, S25–S28 (1993).
- J. E. Muller *et al.*, *N. Engl. J. Med.* **313**, 1315–1322 (1985).
- R. H. Straub, M. Cutolo, *Arthritis Rheum.* **56**, 399–408 (2007).

Acknowledgments: We thank members of the Chawla laboratory and A. Loh for comments on the manuscript. The data presented in this paper are tabulated in the main paper and in the supplementary materials. The authors' work was supported by NIH grants HL076746 and DK094641, a Larry L. Hillblom Foundation Network grant, the Diabetes Family Fund [University of California, San Francisco (UCSF)], and an NIH Director's Pioneer Award (DP1AR064158) to A.C. and NIH grant PO1AI063302 to J.S.C. K.D.N. was supported by Stanford Graduate and American Heart Association (AHA) Predoctoral fellowships, Y.Q. by an AHA Postdoctoral Fellowship, and S.J.F. by an NIH Training Grant (T32AI007334). All animal care and procedures were performed in accordance with Stanford University's Administrative Panel on Laboratory Animal Care and UCSF's Institutional Animal Care and Use Committee guidelines. The authors declare that they have no competing financial interests.

Supplementary Materials

www.sciencemag.org/content/341/6153/1483/suppl/DC1
Materials and Methods
Figs. S1 to S23
Tables S1 and S2
References (38–40)

16 May 2013; accepted 30 July 2013
Published online 22 August 2013;
10.1126/science.1240636

In Situ Observations of Interstellar Plasma with Voyager 1

D. A. Gurnett,^{1*} W. S. Kurth,¹ L. F. Burlaga,² N. F. Ness³

Launched over 35 years ago, Voyagers 1 and 2 are on an epic journey outward from the Sun to reach the boundary between the solar plasma and the much cooler interstellar medium. The boundary, called the heliopause, is expected to be marked by a large increase in plasma density, from about 0.002 per cubic centimeter (cm^{-3}) in the outer heliosphere, to about 0.1 cm^{-3} in the interstellar medium. On 9 April 2013, the Voyager 1 plasma wave instrument began detecting locally generated electron plasma oscillations at a frequency of about 2.6 kilohertz. This oscillation frequency corresponds to an electron density of about 0.08 cm^{-3} , very close to the value expected in the interstellar medium. These and other observations provide strong evidence that Voyager 1 has crossed the heliopause into the nearby interstellar plasma.

As the Sun moves through the interstellar medium, the solar wind plasma flowing outward from the Sun is expected to form a bullet-shaped boundary, the heliopause (1, 2), that separates the solar plasma from the much cooler interstellar plasma (fig. S1). Because the solar wind is supersonic, a shock wave, called the termination shock, must form to slow the solar wind to a subsonic speed so that it can be deflected downstream by the interstellar gas pressure. In 2004 and 2007, Voyagers 1 and 2 crossed the termination shock at 94.0 astronomical units [(AU) 1 AU = 1.49×10^8 km] and 83.4 AU, respectively (3–6). Since then, they have been proceeding outward through the heliosheath, which is a region of shock-heated solar plasma between the termination shock and the heliopause.

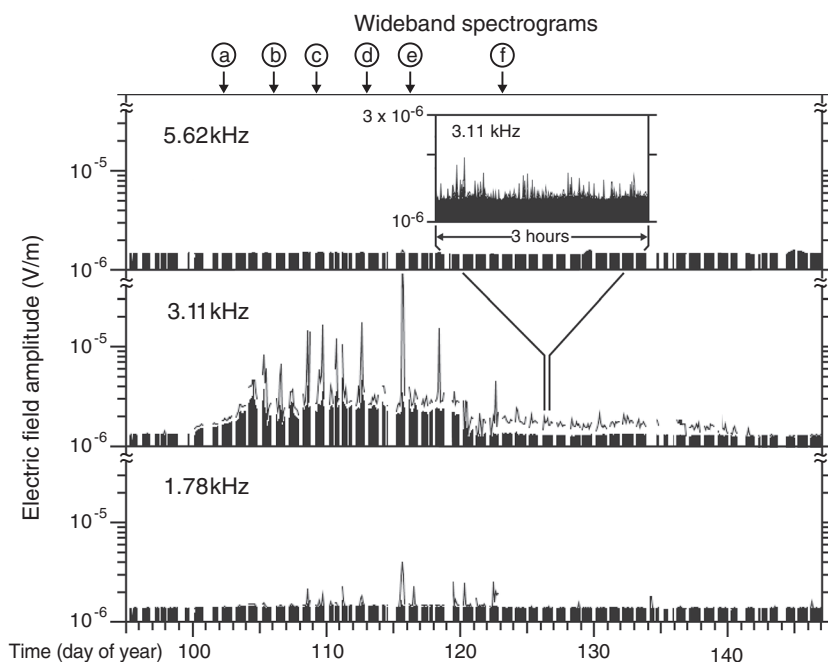
The first indication of a possible encounter with the heliopause was on 28 July 2012, at 121 AU, when the Low Energy Charged Particle (LECP) and Cosmic Ray (CRS) instruments on Voyager 1 detected an abrupt decrease in the intensities of termination shock particles (TSPs) and anomalous cosmic rays (ACRs), and a coincident increase in the galactic cosmic ray (GCR) intensity (7–9). A total of five similar crossings of the boundary were observed, the last being on 25 August 2012, at which time the ACRs decreased to nearly undetectable levels. Because TSPs and ACRs are the dominant energetic charged particles in the heliosheath (10, 11), the decrease in their intensities is consistent with a crossing of the heliopause, as is the increase in the GCR intensity. Although the Magnetometer (MAG) detected closely correlated changes in the magnetic field strength, no appreciable change was observed in the magnetic field direction (12). Because the magnetic field in the local interstellar plasma (13)

is not expected to be in the same direction as in the heliosheath, the absence of a notable change in the magnetic field direction has led to doubts about whether this boundary was the heliopause.

This question could have been resolved had adequate plasma density measurements been available. To maintain pressure balance between the hot ($\sim 10^6$ K) heliosheath plasma (14) and the cool ($\sim 10^4$ K) interstellar plasma (13), a large plasma density increase is expected at the heliopause. Unfortunately, the Plasma (PLS) instrument on Voyager 1 failed in 1980, and the Plasma Wave (PWS) instrument (15), which may have

measured the electron density from the frequency of electron plasma oscillations, detected no oscillations. Electron plasma oscillations occur at a characteristic frequency of the plasma called the electron plasma frequency, $f_p = 8980 \sqrt{n_e}$ Hz, where n_e is the electron number density in cm^{-3} (16). These oscillations are usually excited by electron beams, such as those upstream of interplanetary shocks and after energetic solar electron events. (For a discussion of the mechanism by which electron plasma oscillations are produced, see supplementary text S1.) Electron plasma oscillations were last observed by Voyager 1 in December 2004, upstream of the solar wind termination shock (5).

This situation abruptly changed on 9 April 2013, when the PWS began to detect strong electric field emissions in the 3.11-kHz channel of the onboard spectrum analyzer (Fig. 1). The emissions had the spiky intensity variations characteristic of electron plasma oscillations and continued for almost a month and a half, finally disappearing on 22 May. During this period, we also obtained a series of short 48-s samples of the electric field waveform that were stored on the spacecraft's tape recorder. Using Fourier analysis techniques, we converted these waveforms into frequency-time spectrograms (Fig. 2A). The spectrograms show that the electric field oscillations have a very narrow bandwidth of only a few percent, with an average oscillation frequency of about 2.6 kHz. Using the previously given equation for f_p , this frequency corresponds to an electron density of $n_e = 0.08 \text{ cm}^{-3}$. Careful



Voyager 1, 2013, R = 124 AU, H-Lat = 34.6°, H-Long = 174.2°

Fig. 1. Electric field intensities in the 1.78-, 3.11-, and 5.62-kHz channels of the PWS 16-channel onboard spectrum analyzer. The vertical black regions in each channel give the average field strengths, and the solid lines above give the peak field strengths.

¹University of Iowa, Iowa City, IA 52242, USA. ²NASA–Goddard Space Flight Center (GSFC), Greenbelt, MD 20771, USA. ³The Catholic University of America (CUA), Washington, DC 20064, USA.

*Corresponding author. E-mail: donald-gurnett@uiowa.edu.

measurements of the frequency of the primary component, which can be followed from one spectrogram to the next, show that it increases slowly, at a rate of about 2.6 Hz/day. Sometimes, a second emission line can be seen at a frequency slightly above that of the primary component, as in spectrograms (b) and (f). Such sidebands are a common feature of electron plasma oscillations and can be produced by several processes, including, for example, three-wave parametric decay (17) and trapping of the primary beam-driven oscillations in small density cavities (18).

After examining waveform data recently played back from the spacecraft tape recorder, we found another interval with similar, but much weaker, electron plasma oscillations from 23 October to 27 November 2012 that could not be detected in the onboard spectrum analyzer data (Fig. 2B). The oscillation frequency for this event is about 2.2 kHz, which corresponds to an electron density of about 0.06 cm^{-3} , substantially less than for the April-May 2013 event. As indicated by the sloping dashed white line in Fig. 2B, the change in the oscillation frequency between the two events suggests a smoothly increasing plasma density—i.e., a density ramp—in the region between the two events. In support of this view, the rate of change of the plasma frequency for the dashed white line (2.6 Hz/day) is very close to the rate of change (2.7 Hz/day) given above for the April-May event, and close to that measured for the October-November event, which is slightly lower ($\sim 2.0 \text{ Hz/day}$). At Voyager 1's radial velocity of about 3.5 AU/year, these variations in the plasma frequency correspond to a density gradient of about 19% per AU. A somewhat similar density ramp has been inferred previously from the upward frequency drift of heliospheric 2- to 3-kHz radio emissions (19).

For almost 30 years, the plasma wave instruments on Voyagers 1 and 2 have been detecting transient radio emissions from the outer heliosphere in the frequency range from about 2 to 3 kHz. Two particularly strong events have occurred, the first in 1983–1984 (20), and the second in 1992–1993 (19). It is now generally agreed that these radio emissions are produced near the electron plasma frequency when a strong interplanetary (IP) shock associated with a global merged interaction region reaches the heliopause and interacts with the nearby interstellar plasma. Two components are usually observed, both starting when the IP shock first contacts the heliopause. The first component usually starts at about 2 kHz and gradually increases in frequency with increasing time, eventually reaching about 3 to 3.5 kHz after about half a year. The second component also starts at about 2 kHz and stays at this frequency for a year or more. An example of the radio emission spectrum detected by Voyager 1 during the strong 1992–1993 event is shown in Fig. 3A. In this case, the upward-drifting component is indicated by the sloping white dashed line, increasing in frequency by 1.5 kHz over a period of 231 days. The upward

frequency drift is a common feature of the heliospheric radio emissions and is believed to be caused by an increase in the plasma frequency as the shock propagates into a region of increasing plasma density beyond the heliopause, i.e., a density ramp, possibly caused by a “pileup” of plasma in the region upstream of the heliopause (19), or a plasma “transition region” caused by the interaction with neutral hydrogen, the so-called hydrogen

wall (21). The very prominent constant-frequency component near and slightly above 2 kHz is thought to be either radiation trapped in the low-density heliospheric cavity, or generated beyond the flanks of the heliopause where there is little or no pileup of plasma.

In Fig. 2B, we suggested (as indicated by the sloping white dashed line) that the spacecraft is passing through a smoothly increasing plasma

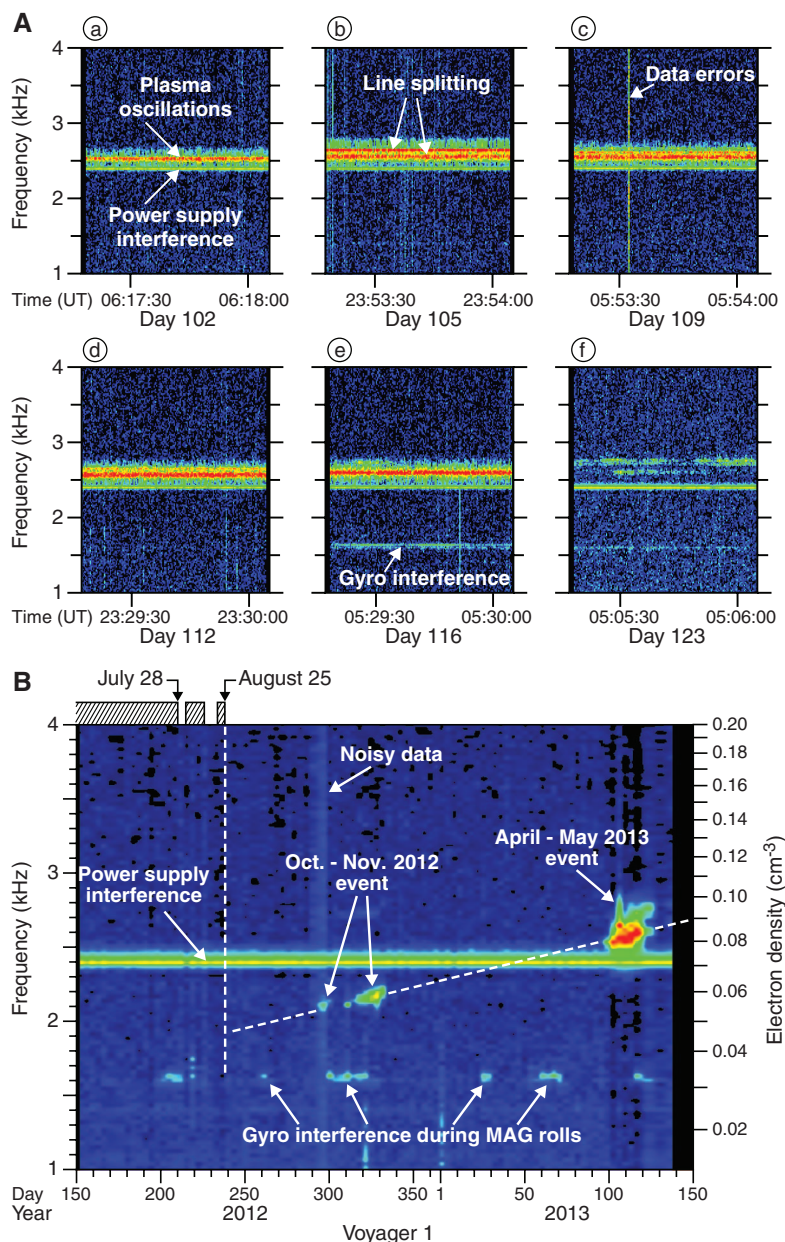


Fig. 2. High-resolution spectrograms. (A) Six selected 48-s frequency-time spectrograms computed from the electric field waveform for the times labeled “a” through “f” in Fig. 1. These data are recorded about twice per week on the spacecraft tape recorder. (B) A composite spectrogram, constructed from spectrograms similar to those in (A), extending over a period of 1 year, starting on day 150, 29 May 2012. In addition to the strong electron plasma oscillation event in the April-May 2013 time period, a much weaker event can be seen at a frequency of about 2.2 kHz in October-November 2012. An electron density scale is given on the right. The vertical dashed white line denotes the last increase in GCRs on 25 August 2012. The sloping dashed white line suggests a density ramp in the region between the two plasma oscillation events.

density—i.e., a density ramp—as it moves outward from the Sun. Radio direction-finding measurements (22) show that the 1992–1993 radio emission originated very close to the region where Voyager 1 is currently located, within about 10° to 15° as viewed from the Sun. Therefore, we investigated whether the density ramp reported here corresponds quantitatively to the density ramp in-

ferred remotely from of the upward-drifting frequency component of the heliospheric radio emissions. To test this hypothesis, we have replotted Fig. 2B in Fig. 3B, with the time of the increase in the GCR intensity on 25 August lined up with the onset of the radio emission in Fig. 3A. In addition, we have adjusted the time scale such that the density ramps in the two plots have

the same slope. When the density ramp in Fig. 3B is then extrapolated backward in time to the point where the increase in the GCR intensity occurs, the plasma frequency is 1.9 kHz, almost exactly the same frequency as the onset of the radio emission in Fig. 3A. Despite the obvious assumptions involved with this extrapolation, this coincidence provides strong support for the view that the GCR intensity increase on 25 August was at the heliopause.

A major unknown factor involved in the interpretation of the upward-drifting radio emission in Fig. 3A is the propagation speed of the IP shock. To estimate the shock speed, we extrapolate the density ramp in Fig. 3B into the future, to the point at which the plasma frequency has increased by 1.5 kHz, to about 3.4 kHz, the same frequency increase as in Fig. 3A. We see that it will take 542 days from the time of the increase in the GCR intensity to reach this point. At the current rate that Voyager 1 is moving outward from the Sun, 3.58 AU/year, this corresponds to a change in radial distance of 5.3 AU. For the density ramps to have the same radial gradient, one can see from Fig. 3A that it would take 231 days for the IP shock to propagate a comparable radial distance, namely 5.3 AU. The shock responsible for the radio emission would then have to propagate outward at a rate of 5.3 AU/231 days, which corresponds to ~ 40 km/s. This is a very plausible shock propagation speed, comparable to those obtained from plasma simulations of IP disturbances propagating into the nearby interstellar medium by Zank and Müller (21) and Washimi *et al.* (23). These comparisons provide strong support for the view that the density ramp inferred from the plasma oscillation events reported here, and the density ramp inferred from the 1992–1993 heliospheric radio emission event, are caused by the same basic density structure on the upstream side of the heliopause, and that the GCR intensity increase on 25 August 2012 marked the crossing of Voyager 1 into the interstellar plasma.

Because electron plasma oscillations are known to be driven by electron beams, we have searched

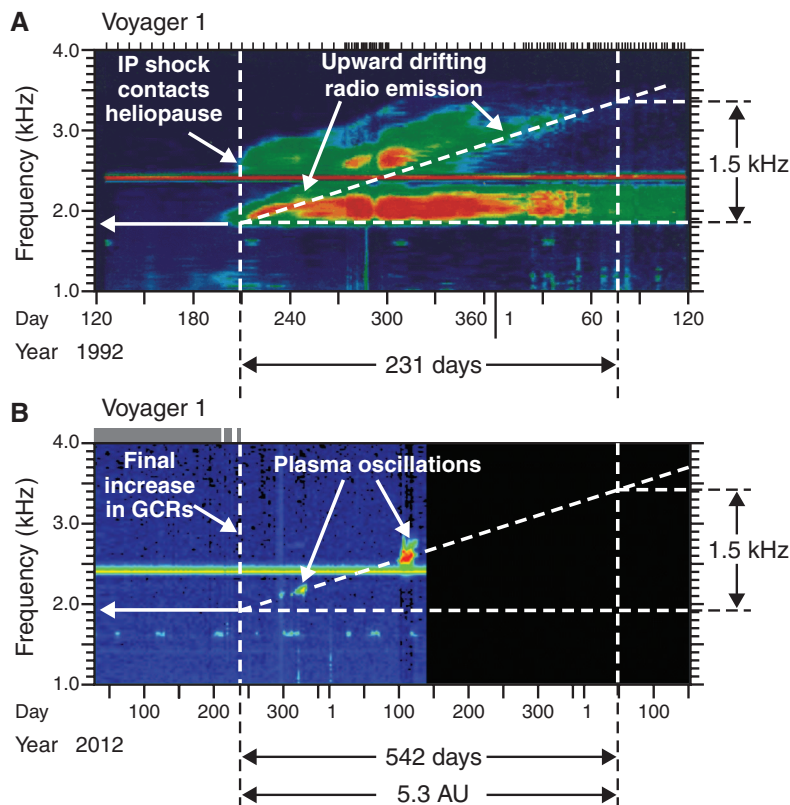
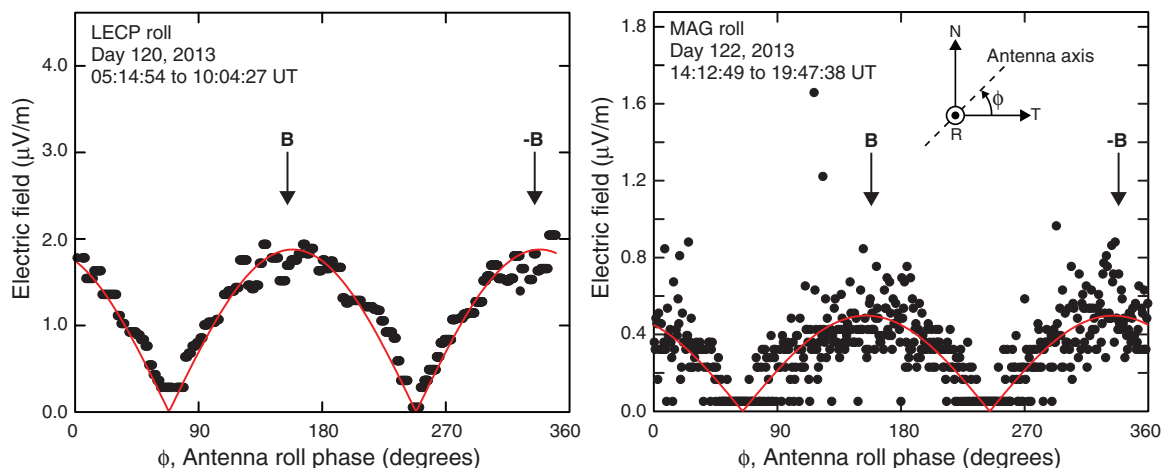


Fig. 3. Comparison of heliospheric 2- to 3-kHz radio emissions to local plasma oscillations. (A) A frequency-time spectrogram of the 1992–1993 heliospheric radio emission event detected remotely by Voyager 1 (19), and **(B)** a rescaled spectrogram of the plasma oscillation frequencies given in Fig. 2B. To facilitate a comparison, the time scales in the two spectrograms have been adjusted so that the white dashed lines have the same slope.

Fig. 4. Electric field polarization.

Plots showing the electric field strength as a function of the roll angle, ϕ , for two spacecraft roll maneuvers. The rolls were around the spacecraft Z axis, which is aligned along the Earth-spacecraft line (i.e., very close to the Sun-spacecraft line) and perpendicular to the electric antenna axis (fig. S2). In both cases, a very pronounced modulation was observed, with two nulls per rotation. Magnetic field measurements during both roll maneuvers, indicated by **B** and **-B**, show that the component of **B** in the roll-plane is within 3° of the electric field direction projected onto the roll-plane. For further details, see supplementary text S2.



both roll maneuvers, indicated by **B** and **-B**, show that the component of **B** in the roll-plane is within 3° of the electric field direction projected onto the roll-plane. For further details, see supplementary text S2.

with the LECP team for evidence of an electron beam around the times of these observations. No electron beam was found, which is not surprising given that the lowest electron energy that can be detected by the LECP is quite high, ~ 28 keV. Often, beams responsible for electron plasma oscillations are at much lower energies. However, a possible causative event was identified in the GCR proton intensities starting on days 80 to 95, 2013 (9), close to the start of the electron plasma oscillations. This event likely originated from a period of extraordinary solar activity beginning early on 5 March 2012, the so-called St. Patrick's day solar storms (24). This timing agrees well with the interplanetary shock model proposed to explain the generation of heliospheric 2- to 3-kHz radio emissions via mode conversion from electron plasma oscillations (19). In the present case, no radio emission could be identified, probably because the plasma oscillations, although strong, are not at the very high field strengths (10 to 100 mV/m) typically associated with the generation of IP radio emissions (25). However, a test can be performed to show consistency with a beam source. For an electrostatic wave, such as an electron plasma oscillation, the electric field $\mathbf{E}$ must be parallel to the wave vector $\mathbf{k}$, which, if driven by a field-aligned electron beam, should be aligned along the magnetic field. We have performed this test using two spacecraft roll maneuvers (Fig. 4). Both showed a very clear modulation in the electric field amplitude with two nulls per rotation, consistent with a linearly polarized electrostatic wave. Magnetic field measurements during both roll maneuvers showed that the component of the magnetic field in the roll plane is within 3° of the electric field direction, as expected.

Here, we have shown that the densities obtained from the recently observed electron plasma oscillations range from 0.06 to 0.08 cm^{-3} , gradually increasing with increasing radial distance at a rate of about 19% per AU. These densities are in close agreement with remote-sensing measurements of plasma densities in the interstellar medium (0.05 to 0.22 cm^{-3}) (13) and much greater than those in the heliosheath (~ 0.001 to 0.003 cm^{-3}), based on Voyager 2 PLS measurements out to its current position at 101 AU (26, 27). Numerous computer simulations also show that the plasma densities remain at about this level throughout the heliosheath (28–30). The reason for the extremely low densities in the heliosheath is that as the solar wind expands outward from the Sun, the density decreases greatly, to $\sim 0.001 \text{ cm}^{-3}$, ahead of the termination shock (5, 6, 31). Although the plasma is compressed by about a factor of 2 at the termination shock (31), once the flow is subsonic, there is no known way to compress the plasma to such high densities. An interplanetary shock can only produce a factor of 4 compression, whereas the heliosheath plasma would have to be compressed by a factor of ≥ 30 to reach the much higher densities reported here. These results, and comparison with previous heliospheric radio measurements,

strongly support the view that Voyager 1 crossed the heliopause into the interstellar plasma on or about 25 August 2012.

The above conclusions assume that there is a single well-defined boundary, the heliopause, that separates the solar plasma from the interstellar plasma, with no linkage between their magnetic fields. The apparent conflict between our conclusion, and the absence of a change in the magnetic field direction (12), puts the above simplified picture into doubt. For example, the interstellar magnetic field may be linked to the solar magnetic field through an as-yet incompletely understood mechanism, such as magnetic flux tube interchange (9), magnetic reconnection (32), or the Kelvin-Helmholtz instability (33). Under such conditions, the very definition of the heliopause comes into question.

References and Notes

- W. I. Axford, in *Physics of the Outer Heliosphere*, S. Grzedzielski, D. E. Page, Eds. (Pergamon, Oxford, 1990), pp. 7–15.
- G. P. Zank, *Space Sci. Rev.* **89**, 413–488 (1999).
- L. F. Burlaga *et al.*, *Science* **309**, 2027–2029 (2005).
- L. F. Burlaga *et al.*, *Nature* **454**, 75–77 (2008).
- D. A. Gurnett, W. S. Kurth, *Science* **309**, 2025–2027 (2005).
- D. A. Gurnett, W. S. Kurth, *Nature* **454**, 78–80 (2008).
- W. R. Webber, F. B. McDonald, *Geophys. Res. Lett.* **40**, 1665–1668 (2013).
- E. C. Stone *et al.*, *Science* **341**, 150–153 (2013).
- S. M. Krimigis *et al.*, *Science* **341**, 144–147 (2013).
- E. C. Stone *et al.*, *Science* **309**, 2017–2020 (2005).
- R. B. Decker *et al.*, *Science* **309**, 2020–2024 (2005).
- L. F. Burlaga, N. F. Ness, E. C. Stone, *Science* **341**, 147–150 (2013).
- P. C. Frisch, S. Redfield, J. D. Slavin, *Annu. Rev. Astron. Astrophys.* **49**, 237–279 (2011).
- D. J. McComas *et al.*, *Geophys. Res. Lett.* **38**, L18101 (2011).
- F. L. Scarf, D. A. Gurnett, *Space Sci. Rev.* **21**, 289 (1977).
- D. A. Gurnett, A. Bhattacharjee, in *Introduction to Plasma Physics with Space and Laboratory Applications* (Cambridge Univ. Press, Cambridge, 2005), p. 11.
- I. H. Cairns, P. A. Robinson, *Geophys. Res. Lett.* **19**, 2187–2190 (1992).
- R. E. Ergun *et al.*, *Phys. Rev. Lett.* **101**, 051101 (2008).
- D. A. Gurnett, W. S. Kurth, S. C. Allendorf, R. L. Poynter, *Science* **262**, 199–203 (1993).
- W. S. Kurth, D. A. Gurnett, F. L. Scarf, R. L. Poynter, *Nature* **312**, 27–31 (1984).
- G. P. Zank, H.-R. Müller, *J. Geophys. Res.* **108**, 1240 (2003).
- W. S. Kurth, D. A. Gurnett, *J. Geophys. Res.* **108**, 8027 (2003).
- H. Washimi *et al.*, *Astrophys. J.* **757**, L2 (2012).
- M. Guhathakurta, *Eos* **94**, 165–166 (2013).
- S. D. Bale *et al.*, *Geophys. Res. Lett.* **26**, 1573–1576 (1999).
- J. D. Richardson, C. Wang, *Astrophys. J.* **759**, L19 (2012).
- <http://web.mit.edu/afs/athena/org/space/www/voyager.html>
- G. P. Zank *et al.*, *Astrophys. J.* **763**, 20 (2013).
- N. V. Pogorelov, G. P. Zank, T. Ogino, *Astrophys. J.* **644**, 1299–1316 (2006).
- H. Washimi *et al.*, *Mon. Not. R. Astron. Soc.* **416**, 1475–1485 (2011).
- J. D. Richardson, J. C. Kasper, C. Wang, J. W. Belcher, A. J. Lazarus, *Nature* **454**, 63–66 (2008).
- J. F. Drake, M. Opher, M. Swisdak, J. N. Chamoun, *Astrophys. J.* **709**, 963 (2010).
- V. Florinski, G. P. Zank, N. V. Pogorelov, *J. Geophys. Res.* **110**, A07104 (2005).

Acknowledgments: We thank E. C. Stone, S. M. Krimigis, and J. D. Richardson, for their helpful comments. We also thank R. B. Decker, L. J. Granroth, J. C. Hall, A. Persoon, O. Santolík, and R. F. Wong for help in various data processing issues. The research at Iowa was supported by NASA through contract 1415150 with the JPL. The research at GSFC was supported by NASA contract NNG11PM48P, and the research at CUA was supported in part by NASA grant NNX12AC63G.

Supplemental Materials

www.sciencemag.org/content/341/6153/1489/suppl/DC1
Supplementary Text
Figs. S1 and S2
References (34–36)

10 June 2013; accepted 16 August 2013
Published online 12 September 2013;
10.1126/science.1241681

Distances, Luminosities, and Temperatures of the Coldest Known Substellar Objects

Trent J. Dupuy^{1*} and Adam L. Kraus^{1,2}

The coolest known brown dwarfs are our best analogs to extrasolar gas-giant planets. The prolific detections of such cold substellar objects in the past 2 years have spurred intensive follow-up, but the lack of accurate distances is a key gap in our understanding. We present a large sample of precise distances based on homogeneous mid-infrared astrometry that robustly establishes absolute fluxes, luminosities, and temperatures. The coolest brown dwarfs have temperatures of 400 to 450 kelvin and masses almost equal to 5 to 20 times that of Jupiter, showing they bridge the gap between hotter brown dwarfs and gas-giant planets. At these extremes, spectral energy distributions no longer follow a simple correspondence with temperature, suggesting an increasing role of other physical parameters, such as surface gravity, vertical mixing, clouds, and metallicity.

One major goal in astrophysics is to extend previous successes in the characterization and modeling of stellar atmospheres

to the much cooler atmospheres of extrasolar planets. A key pathway is the identification of free-floating objects that share not only common

temperatures but also common masses and thus surface gravities with exoplanets. In recent years, searches for ever colder free-floating brown dwarfs, objects with masses below the hydrogen-fusing mass limit, have steadily pushed the census of the solar neighborhood to ever lower masses and finally perhaps into the planetary-mass regime ($\lesssim 13$ Jupiter masses).

The detection of large samples of brown dwarfs at the beginning of the last decade ushered in two, now widely accepted, spectral types denoted by the letters “L” and “T” that extend the canonical OBAFGKM scheme for classifying stars that had stood untouched for nearly a century. Over the past 2 years, candidates for a “Y” spectral class have been uncovered in binary surveys (1, 2) and in all-sky imaging data from WISE, the Wide-Field Infrared Survey Explorer (3). The primary criterion adopted to trigger this class has been the appearance of ammonia (NH_3) absorption in near-infrared (1 to 2.5 μm) spectra.

Y dwarfs probe colder atmospheric physics than before, with putative effective temperatures as low as $T_{\text{eff}} \sim 300$ K and masses of ≈ 5 to 20 Jupiter masses (3). If found orbiting a star, a Y dwarf would likely be considered a gas-giant planet. However, these estimated properties of Y dwarfs are speculative given the uncertainty in their temperatures, ages, and luminosities. Temperatures have only been estimated from model atmospheres that use incomplete molecular line lists and simple prescriptions for complex processes like nonequilibrium chemistry and condensate formation.

An independent approach for determining temperatures is to combine bolometric luminosities (L_{bol}) with evolutionary model-predicted radii ($R_{\star}$) and apply the Stefan-Boltzmann Law, $T_{\text{eff}} \equiv (4\pi\sigma R_{\star}^2/L_{\text{bol}})^{-1/4}$. Recent observations of transiting substellar objects generally support evolutionary model radius predictions over a wide range of masses (4–6). Although many may not be ideal test cases because they may have formed via core accretion or have been intensely irradiated, variations in radii are expected to be relatively small and not strongly influence our resulting temperatures given the weak dependence on radius ($T_{\text{eff}} \propto R_{\star}^{-1/2}$). Therefore, the key measurements needed to determine temperatures via luminosity are accurate distances to Y dwarfs, along with a method for computing L_{bol} from multiwavelength photometry.

Trigonometric parallaxes provide the only direct means of measuring distances to stars. A star’s distance is inversely proportional to the amplitude of its apparent periodic motion on the sky relative to more distant background stars, which is due to Earth’s orbital motion

around the Sun. The amplitude of this effect is small, 0.1 arc sec for a star at 10 parsec, and thus measuring parallaxes requires long-term, precise position measurements. We have been using the Infrared Array Camera (IRAC) on board the Spitzer Space Telescope to obtain such astrometry of late-T and Y dwarfs from 2011 to 2012.

Spitzer currently trails Earth by ≈ 2 months in its solar orbit, and keeping its solar shield directed at the Sun forces the telescope to observe stars near parallax maximum. By maintaining a cold temperature, Spitzer can obtain sensitive images in the thermal mid-infrared, where Y dwarfs emit most of their flux, giving it an advantage over ground-based near-infrared observations of Y dwarfs. We also use an improved correction for the nonlinear optical distortion of Spitzer/IRAC that enables ~ 10 times smaller residual errors than the correction used by the standard data pipeline, allowing us to unlock the precision astrometric capabilities of Spitzer.

By combining our parallaxes (table S1 and fig. S1) with photometry from the literature (7–9), we have determined absolute magnitudes in the near-infrared $YJHK$ bands (≈ 1.0 to 2.4 μm) and

Spitzer’s mid-infrared bands at 3.6 and 4.5 μm (table S2 and Figs. 1 and 2). For each spectral type bin, we computed the weighted mean absolute magnitude as well as upper or lower limits on the amount of intrinsic scatter in the magnitudes (table S3).

Objects classified as normal Y0 dwarfs are ≈ 2 magnitudes ($\approx$ six times) fainter in the near-infrared compared with the latest type T dwarfs, yet they generally share very similar colors. The most notable exception is that the $Y - J$ colors become much bluer for Y dwarfs (9), which we find is due to flux at ≈ 1.25 μm dropping by a factor of five whereas flux at ≈ 1.05 μm only drops by a factor of 2.5. This behavior is consistent with prior speculation that Y dwarfs may be so cool that the alkali atoms that dominate absorption at blue wavelengths for warmer brown dwarfs finally become locked into molecules like Na_2S and KCl , thereby reducing the opacity at 1.05 μm relative to 1.25 μm (10). The appearance of such molecules could result in the return of substantial condensate clouds (11) and corresponding variability and weather.

In contrast to their near-infrared behavior, Y dwarfs show remarkable diversity in their mid-infrared colors. Even though they are only $\approx$ two

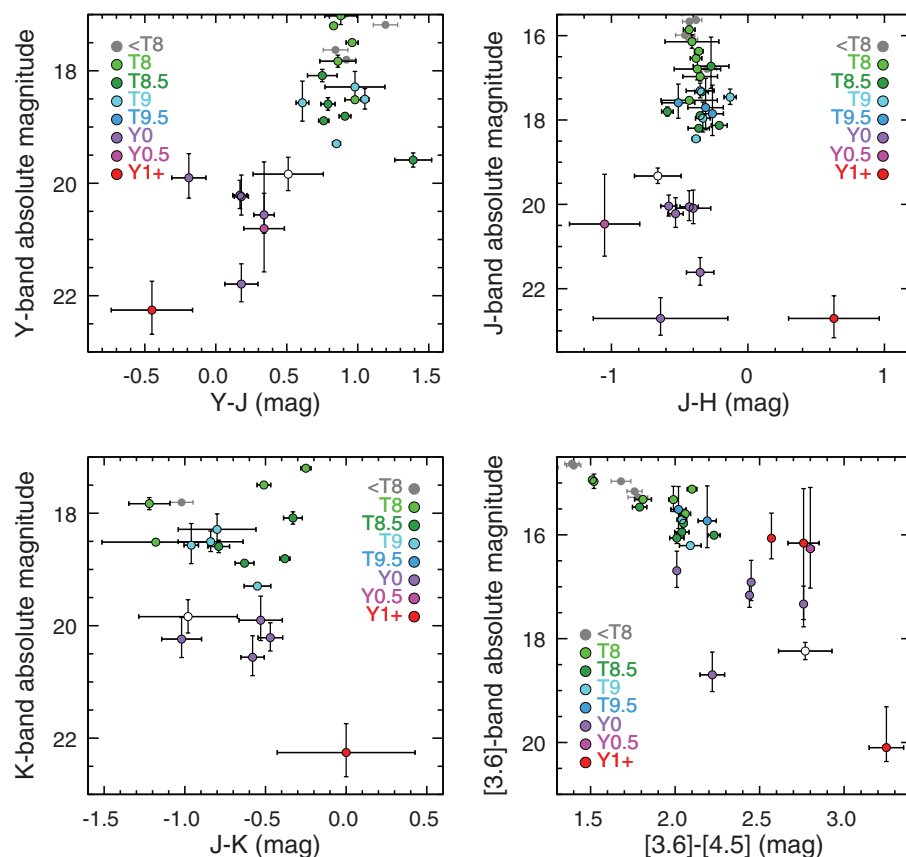


Fig. 1. Color-magnitude diagrams for all objects with spectral types T8 and later that have direct distance measurements. Data points are color-coded according to spectral type, with open/white points indicating that no spectra are available. Small gray points are earlier type field brown dwarfs. Near-infrared photometry is on the Mauna Kea Observatory system. Error bars indicate ± 1 SD.

¹Harvard-Smithsonian Center for Astrophysics, 60 Garden Street, Cambridge, MA 02138, USA. ²Astronomy Department, University of Texas at Austin, 1 University Station C1400, Austin, TX 78712, USA.

*Corresponding author. E-mail: tdupuy@cfa.harvard.edu

times fainter than the latest T dwarfs at these wavelengths, they range from the same color as late-T dwarfs to much redder (≈ 0.8 magnitude). One of the reddest objects is WISEP J1405+5534, which has been typed as “Y0 peculiar?” because its *H*-band spectral peak is shifted 60 Å redder than the Y0 standard, WISEP J1738+2732 (3). We find that WISEP J1405+5534 in fact has a very similar temperature to other Y0 dwarfs (table S5), indicating that its unusual spectrum is due to another physical property. Both the mid-infrared color and peculiar spectrum may be explained by a reduced level of nonequilibrium chemistry in the photosphere, perhaps because of reduced vertical mixing. This would produce enhanced NH_3 absorption at *H* band as compared to other Y0 dwarfs and enhanced CH_4 absorption relative to CO driving WISEP J1405+5534 to redder [3.6] – [4.5] colors.

The coldest brown dwarfs also demonstrate unusual behavior in their absolute fluxes as a function of spectral type. Despite their plummeting near-infrared flux (normal Y0 dwarfs are $\approx$ six times fainter than the latest T dwarfs), Y0

dwarfs have indistinguishable fluxes compared to each other to within 15 to 25%. This is very unusual compared with warmer brown dwarfs, which do not show such step-function behavior at any spectral type transition and also show much larger intrinsic scatter (≈ 30 to 50%) in absolute fluxes for a given spectral type (12). This homogeneity among the Y0 dwarfs is further unexpected because it reverses the trend observed for the late-T dwarfs that the scatter increases substantially with later type, cooler objects (Fig. 3). For example, here we double the sample of T9 dwarfs with accurate distances and find that their near-infrared fluxes typically have a scatter of 130 to 210%.

Another unexpected result is that T9.5 dwarfs appear to be brighter at all bandpasses than the mean for T9 dwarfs and the T9 standard UGPS J0722–0540. Given the smaller sample of T9.5 dwarfs (three objects) and their more uncertain distances, this brightening is currently a 2σ result; that is, the weighted means in table S3 are consistent with being equal at a P value of 0.05. Such a brightening is reminiscent of the change in

near-infrared fluxes from late-L to early-T dwarfs (13, 14); however, we note that the brightening at the L/T transition only occurs at blue near-infrared wavelengths, whereas we see brightening at all bands for the T9.5 dwarfs.

To derive bolometric luminosities from the absolute fluxes, we computed “supermagnitudes” by summing the fluxes in near- and mid-infrared bandpasses. This is an approximation to the standard method of integrating the observed spectral energy distribution as a function of wavelength, which is not possible for Y dwarfs given the current lack of sensitive mid-infrared spectrographs. We derive a multiplicative correction to account for the remaining flux not captured in these bands from a large grid of model atmospheres (11, 15). The weak dependence on these models is highlighted by the 8% fractional uncertainty in this correction factor (fig. S4).

We used the Cond evolutionary models (16) to estimate radii and thereby temperatures, masses, and surface gravities from the bolometric luminosities of our sample (Fig. 4 and table S5). We assumed fiducial ages of 1 and 5 billion years as expected for the field population (17, 18). The tangential velocities for our sample are consistent with having such typical ages. We find that the fractional change in temperature over this narrow range of spectral types is quite large: The mean temperature of T8 dwarfs is 685 to 745 K (for 1 to 5 Gyr), and this drops to 410 to 440 K for Y0 dwarfs (table S6). Thus, these two subtypes alone span the same fractional range in temperature as the entire sequence of FGK stars (7300 to 4400 K) that are ~ 10 times hotter.

Although much cooler than their late-T counterparts, Y0 dwarfs turn out to be significantly warmer than previously suggested from model atmosphere fitting (fig. S5). The most common best-fit models of Y0 dwarfs in previous work have $T_{\text{eff}} = 350$ K, with plausible model fits of 400 K in some cases (3). Thus, model fits are typically 60 to 90 K (≈ 15 to 25%) cooler than we find from our distances combined with evolutionary model radii. If the fault lies with our assumed radii, they would need to be 30 to 50% larger than expected because $T_{\text{eff}} \propto R_{\star}^{-1/2}$. This would require very young ages (≤ 100 million years)

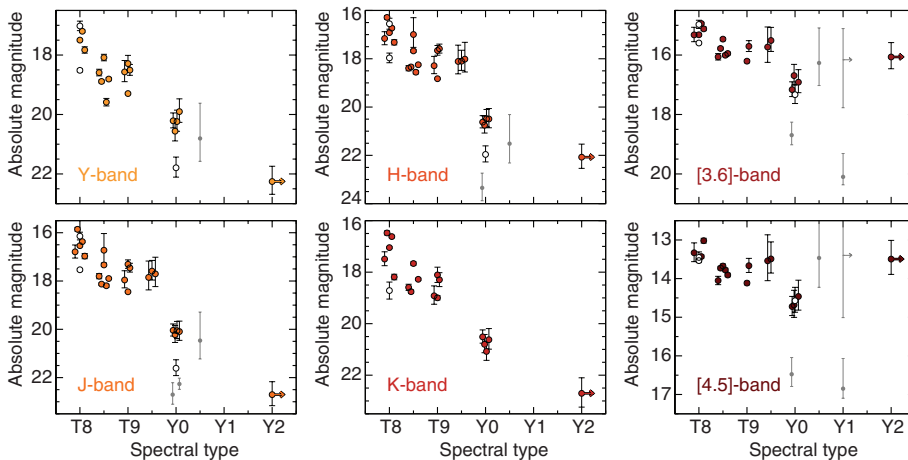
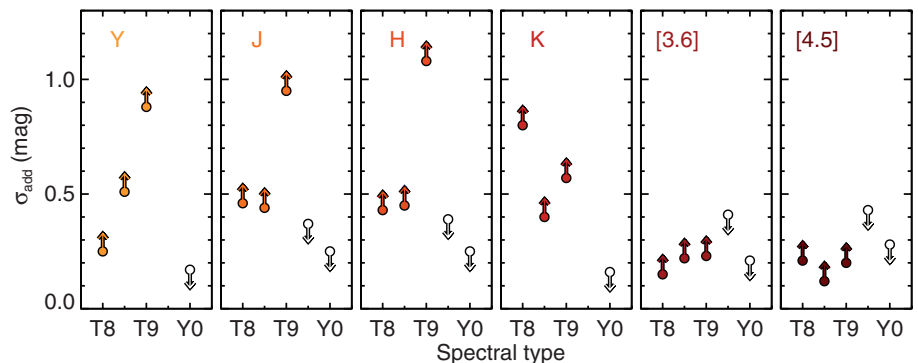


Fig. 2. Absolute magnitude as a function of spectral type for near-infrared and mid-infrared bandpasses. Objects typed as peculiar are shown as open white symbols. Objects with very uncertain distances are plotted with smaller gray symbols. Error bars for spectral types are not plotted, and small x-axis offsets have been added to the spectral types for clarity.

Fig. 3. Intrinsic scatter in absolute magnitudes as a function of spectral type. Colored symbols indicate lower limits, and open symbols show upper limits where no intrinsic scatter is detected. Y0 dwarfs show very low intrinsic scatter in the near-infrared, reversing the trend observed at the end of the T dwarf sequence that later type T dwarfs show increasing dispersion in their near-infrared absolute magnitudes. The much smaller sample of T9.5 dwarfs are also suggestive of this reversal at *J* and *H* bands. There is no evidence for such a reversal in the mid-infrared, where both late-T and Y dwarfs show much less intrinsic scatter and distance uncertainties for Y dwarfs do not permit strong enough upper limits.



or very large systematic errors in the evolutionary models that are not likely given the aforementioned empirical validation from transiting brown dwarfs. Rather, we suggest that parameters derived from fitting model atmospheres to near-infrared spectra, where 5% of the flux emerges, are less likely to be accurate because current atmospheres imperfectly reproduce observed spectra.

By using our luminosity measurements, we find that the coldest brown dwarfs would be 6 to 10 Jupiter masses given an age of 1 Gyear. An older age of 5 Gyear implies 16 to 25 Jupiter masses. These masses therefore straddle the current demarcation of “planetary mass” set by the deuterium-fusing mass limit of ≈ 13 Jupiter masses (19–21). Thus, it is possible that the at-

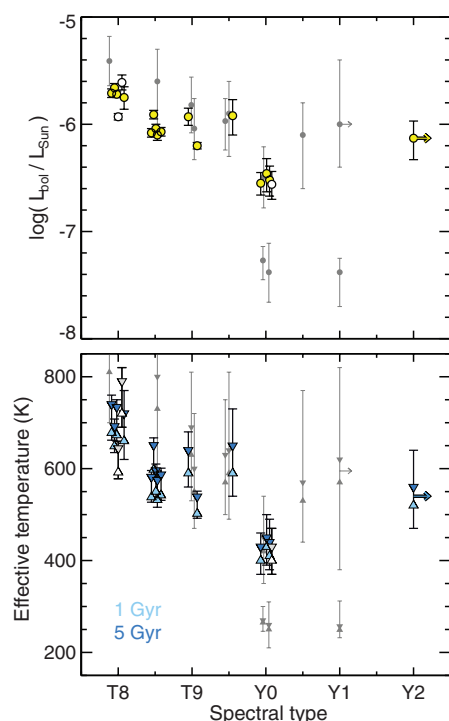


Fig. 4. Bolometric luminosities (L_{bol}) and effective temperatures for objects of spectral type T8 and later. Spectrally peculiar objects are denoted by white symbols, and objects with L_{bol} uncertainties larger than 0.2 dex are shown as smaller, gray symbols. These are either objects with very uncertain distances or the components of tight binaries, where the lack of resolved mid-infrared photometry results in a very uncertain bolometric flux. Error bars for spectral types are not plotted, and small x-axis offsets have been added to the spectral types for clarity. Effective temperatures are derived from our L_{bol} measurements and Cond evolutionary model radii. Upward and downward pointing triangles correspond to the median L_{bol} and lower and upper age limits used (see table S5). Error bars show the range of temperatures corresponding to the $\pm 1\sigma$ range of L_{bol} over the same age range.

mospheres of our objects harbor deuterated molecules such as HDO or CH_3D that have not yet been detected because of the observational challenges (22).

Given the interest in both identifying the coldest atmospheric benchmarks and searching for the bottom of the initial mass function, we briefly consider the most extreme objects in our sample in terms of temperature and mass. WISEP J1828+2650 has been dubbed the archetypal Y dwarf with a model-atmosphere temperature of $\lesssim 300$ K, that is, room temperature, based on extremely red colors implying that the Wien tail of its underlying blackbody distribution has moved into the near-infrared (3). Our luminosity for this object is inconsistent with such a low temperature, and we find it must be at least 420 K at 2σ ; our calculations give 520^{+60}_{-50} K at an age of 1 Gyear. [If WISEP J1828+2650 is a binary as proposed by (9), the 2σ limit at 1 Gyear only drops to 360 and 340 K for the hypothetical two components.] Its atypical properties compared with other Y dwarfs may simply be due to a slightly lower surface gravity and thereby slightly younger age, which qualitatively agrees with model predictions that the collapse in near-infrared flux happens at warmer temperatures for lower surface gravity (23). Ross 458C is a contender for the lowest mass object, at 7 Jupiter masses, if its age is near the 150-Myr lower limit of its proposed age range (24). However, WD 0806–661B is the most secure case for both lowest temperature (330 to 375 K) and lowest mass (6 to 10 Jupiter masses) object known, given that it has a precise age of 2.0 ± 0.5 Gyear (25).

Overall, our results strengthen the connection between the coolest brown dwarfs and gas-giant exoplanets. We validate that they probe an extreme physical regime that bridges the gap between previously known, hotter brown dwarfs and Jupiter-like planets. We find that objects of very similar temperatures can have widely varying spectral energy distributions and absorption features, for example, mid-infrared colors vary by 0.8 magnitude for the same T_{eff} . Along with the fact that the $\geq Y2$ dwarf is warmer than the Y0 dwarfs, this implies that temperature is not the principal determinant in shaping spectra but rather seems to be on comparable footing with other physical properties, such as surface gravity, vertical mixing, clouds, and perhaps metallicity. Consequently, the current spectral classification scheme used to identify Y dwarfs may not strongly correlate with temperature as it generally does for L and T dwarfs. This could explain the unusually homogeneous fluxes for Y0 dwarfs, unusually heterogeneous fluxes for T9 dwarfs, and plateau or brightening of flux from T9 to T9.5.

References and Notes

1. K. L. Luhman, A. J. Burgasser, J. J. Bochanski, *Astrophys. J.* **730**, L9 (2011).
2. M. C. Liu *et al.*, *Astrophys. J.* **740**, 108 (2011).

3. M. C. Cushing *et al.*, *Astrophys. J.* **743**, 50 (2011).
4. M. Deleuil *et al.*, *Astron. Astrophys.* **491**, 889–897 (2008).
5. C. Hellier *et al.*, *Nature* **460**, 1098–1100 (2009).
6. D. R. Anderson *et al.*, *Astrophys. J.* **726**, L19 (2011).
7. J. D. Kirkpatrick *et al.*, *Astrophys. J. Suppl. Ser.* **197**, 19 (2011).
8. J. D. Kirkpatrick *et al.*, *Astrophys. J.* **753**, 156 (2012).
9. S. K. Leggett *et al.*, *Astrophys. J.* **763**, 130 (2013).
10. M. C. Liu, T. J. Dupuy, B. P. Bowler, S. K. Leggett, W. M. J. Best, *Astrophys. J.* **758**, 57 (2012).
11. C. V. Morley *et al.*, *Astrophys. J.* **756**, 172 (2012).
12. T. J. Dupuy, M. C. Liu, *Astrophys. J. Suppl. Ser.* **201**, 19 (2012).
13. A. J. Burgasser *et al.*, *Astrophys. J. Suppl. Ser.* **166**, 585–612 (2006).
14. M. C. Liu *et al.*, *Astrophys. J.* **647**, 1393–1404 (2006).
15. D. Saumon, M. S. Marley, M. Abel, L. Frommhold, R. S. Freedman, *Astrophys. J.* **750**, 74 (2012).
16. I. Baraffe, G. Chabrier, T. S. Barman, F. Allard, P. H. Hauschildt, *Astron. Astrophys.* **402**, 701–712 (2003).
17. A. J. Burgasser, *Astrophys. J. Suppl. Ser.* **155**, 191–207 (2004).
18. P. R. Allen, D. W. Koerner, I. N. Reid, D. E. Trilling, *Astrophys. J.* **625**, 385–397 (2005).
19. D. Saumon *et al.*, *Astrophys. J.* **460**, 993 (1996).
20. G. Chabrier, I. Baraffe, *Annu. Rev. Astron. Astrophys.* **38**, 337–377 (2000).
21. P. Mollière, C. Mordasini, *Astron. Astrophys.* **547**, A105 (2012).
22. G. Chabrier, I. Baraffe, F. Allard, P. Hauschildt, *Astrophys. J.* **542**, L119–L122 (2000).
23. A. Burrows, D. Sudarsky, J. I. Lunine, *Astrophys. J.* **596**, 587–596 (2003).
24. A. J. Burgasser *et al.*, *Astrophys. J.* **725**, 1405–1420 (2010).
25. K. L. Luhman *et al.*, *Astrophys. J.* **744**, 135 (2012).

Acknowledgments: We thank M. C. Liu for many helpful suggestions for improving this article; C. V. Morley for fruitful discussions regarding her and collaborators’ model atmospheres, particularly the Vega zero points; K. N. Allers for assistance in computing photometry from our IRAC data; and M. C. Cushing for making available published spectra of our targets. This work is based on observations made with the Spitzer Space Telescope, which is operated by the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA. T.J.D. acknowledges support from Hubble Fellowship grant HST-HF-51271.01-A awarded by the Space Telescope Science Institute, which is operated by the Association of Universities for Research in Astronomy for NASA, under contract NAS 5-26555. A.L.K. is supported by a Clay Fellowship. Our imaging data are available in the Spitzer Heritage Archive at <http://sha.ipac.caltech.edu/applications/Spitzer/SHA>, and the astrometric measurements we derived are given in table S1.

Supplementary Materials

www.sciencemag.org/content/341/6153/1492/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S5
Tables S1 to S6
References (26–68)

13 June 2013; accepted 23 August 2013
Published online 5 September 2013;
10.1126/science.1241917

Observation of Dirac Node Formation and Mass Acquisition in a Topological Crystalline Insulator

Yoshinori Okada,^{1,2} Maksym Serbyn,^{3*} Hsin Lin,^{4*} Daniel Walkup,¹ Wenwen Zhou,¹ Chetan Dhital,¹ Madhab Neupane,⁵ Suyang Xu,⁵ Yung Jui Wang,⁴ R. Sankar,⁶ Fangcheng Chou,⁶ Arun Bansil,⁴ M. Zahid Hasan,^{5,7} Stephen D. Wilson,¹ Liang Fu,³ Vidya Madhavan^{1†}

In topological crystalline insulators (TCIs), topology and crystal symmetry intertwine to create surface states with distinct characteristics. The breaking of crystal symmetry in TCIs is predicted to impart mass to the massless Dirac fermions. Here, we report high-resolution scanning tunneling microscopy studies of a TCI, $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ that reveal the coexistence of zero-mass Dirac fermions protected by crystal symmetry with massive Dirac fermions consistent with crystal symmetry breaking. In addition, we show two distinct regimes of the Fermi surface topology separated by a Van-Hove singularity at the Lifshitz transition point. Our work paves the way for engineering the Dirac band gap and realizing interaction-driven topological quantum phenomena in TCIs.

Topological crystalline insulators (TCIs) (1–5) are a class of materials that possess a new type of electronic topology that arises from crystalline symmetry; this gives rise to surface states with an unusual band dispersion and spin texture. In particular, the (001)-oriented surfaces of IV–VI semiconductor TCIs (Fig. 1A) harbor two generations of Dirac cones at different energies (Fig. 1B). The band structure of (001) surface states can be visualized by starting from a pair of Dirac points located at the $\bar{X}$ points at the edge of the surface Brillouin zone (BZ), with the Dirac point energies close to the conduction and valence band edge, respectively (6). The hole-branch of the upper Dirac cone and the electron-branch of the lower Dirac cone coexist inside the band gap. The hybridization between these two branches leads to an avoided crossing in all directions except along the $\bar{\Gamma}\bar{X}$ line, where a band crossing is dictated by electronic topology of TCI and protected by the (110) mirror symmetry (6). Such a band reconstruction generates a new pair of low-energy Dirac nodes displaced symmetrically away from each $\bar{X}$ point (Fig. 1B).

The distinctive band structure of the TCI surface states has two important consequences. First, the formation of low-energy Dirac nodes is accompanied by a Lifshitz transition (7): As the

Fermi energy moves deep into the band gap, the Fermi surface changes from concentric pockets of opposite carrier types centered at $\bar{X}$ into a pair of disconnected pockets across the BZ boundary (Fig. 1B). The change of Fermi surface topology occurs via a saddle point in the surface band structure, which has been predicted to lead to a divergence in the electronic density of states known as Van Hove singularity (VHS) at the saddle-point energy (6). Although a VHS generally enhances interaction effects and drives electronic instabilities (8–11), in TCIs interaction effects in combination with band topology may result in novel correlated states (12, 13). Second, when mirror symmetry is broken—either spontaneously or by external perturbations—the TCI surface states acquire a gap, creating massive Dirac fermions and providing an exciting avenue to control the properties of Dirac materials through strain or doping.

We used a low-temperature scanning tunneling microscope (STM) to track the dispersion and the density of states of $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ with a nominal doping of $x = 0.5$ [determined to have an actual doping level of $x = 0.34$ (14)], which lies in the topological regime (5). Single-crystal $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ samples were cleaved in ultrahigh vacuum at ~ 80 K before being inserted into the STM, and all measurements were obtained at ~ 4 K. Although $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ has a three-dimensional (3D) crystal structure (Fig. 1A), it cleaves along the [001] plane, resulting in the Pb/Sn–Se surface shown in Fig. 2A. STM topography reveals a square lattice whose inter-atom spacing of $4.32 \text{ \AA}$ indicates a preferential imaging of either the Pb/Sn or the Se sublattice.

The overall density of states in this TCI can be obtained by measuring dI/dV spectra. A typical $dI/dV(\text{eV})$ spectrum in this material (Fig. 1C) shows a V-shaped density of states, with a minimum at ~ -80 meV. By comparing this with the schematic band structure, we tentatively as-

signed the minimum at -80 meV to the Dirac point deep in the band gap labeled E_{DP1} in Fig. 1D and the approximately symmetric peaks on either side of this Dirac point (at ~ -40 meV and ~ -120 meV) to the VHSs at the saddle point energy, which as we show later in this paper is consistent with our data. To establish the surface-state dispersion, we developed a framework, which combines our magnetic field-dependent STM data with a theoretical model.

It is necessary to first understand the level of inhomogeneity in these samples. To do this, we compared spectra obtained at various spatial locations (Fig. 2C) and found a notable degree of spectral homogeneity over at least $300 \text{ \AA}$, despite the presence of randomly distributed Sn atoms within the Pb lattice; we henceforth used linecut-averaged spectra along the line shown in Fig. 2A for our analysis. This homogeneity should be contrasted with the highly inhomogeneous nature of graphene as well as doped topological insulators. This important feature makes $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ a much more stable host for topological surface states and allows true access to physics at the Dirac point with a variety of experimental probes.

The line-cut averaged spectra at various magnetic fields (Fig. 3A) show clear Landau level peaks. Comparing the zero-field spectrum with the spectra at higher fields, we found that for nonzero magnetic fields, a peak located precisely at the density of states minimum (at ~ -80 meV) emerges. It is nondispersive; its position does not change with magnetic field, which confirms its origin as the 0th LL located at the Dirac point, E_{DP1} . In addition, we found other nondispersing peaks, which have been labeled E_{-}^* , E_{+}^* , and E_{DP2} in Fig. 3A. To understand the electronic structure, as a first step we analyzed the LL data within a semiclassical picture. For normal 2D bands with linear or quadratic dispersion, the semiclassical approximation is applicable, and a plot of the LL peak position as a function of nB (where n is the LL index and B is the magnetic field strength) can be used to obtain information on the dispersion (15–17). However, this requires us to index the dispersing LL peaks, which is a nontrivial task in this material. At energies away from the saddle points, the band structure is characteristic of Dirac fermions, with approximately linear dispersion (6); the peak energies can therefore be expected to collapse to one curve as a function of $\sqrt{nB}$. By using this scaling behavior as a constraint, we obtained the peak index assignments for the dispersing LLs (Figs. 2B and 3B).

The resulting plot of the LL energy with $\sqrt{nB}$ is shown in Fig. 3C. The sharp discontinuity in the LL plot in Fig. 3C and the jump in the LL index from $n = 2$ to 6 occur at the same energy as the dI/dV peak labeled $E_{\text{VHS}+}$ in Fig. 3, A and B, which suggests that they have a similar origin. Consulting the schematic band structure in Fig. 1D suggests that these features are a result of the Lifshitz transition; the flat dispersion at the saddle point creating the VHS as well as the missing peaks ($n = 3, 4, 5$). The jump in index

¹Department of Physics, Boston College, Chestnut Hill, MA 02467, USA. ²World Premier International–Advanced Institute for Materials Research, Tohoku University, Sendai, 980-8577, Japan. ³Department of Physics, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. ⁴Department of Physics, Northeastern University, Boston, MA 02115, USA. ⁵Joseph Henry Laboratory, Department of Physics, Princeton University, Princeton, NJ 08544, USA. ⁶Center for Condensed Matter Sciences, National Taiwan University, Taipei 10617, Taiwan. ⁷Princeton Center for Complex Materials, Princeton University, Princeton, NJ 08544, USA.

*These authors contributed equally to this work.

†Corresponding author. E-mail: madhavan@bc.edu

corresponds exactly to the doubling of the Fermi surface area expected from the Lifshitz transition (Fig. 3E). The observation of VHSs in a topologically nontrivial material close to the Fermi energy opens the exciting possibility of achieving correlated states in a Dirac material. VHSs have

previously been observed in graphene (18); however, unlike in graphene, symmetry-breaking interactions modifying existing topologically protected electronic states in TCIs have the potential of generating helical domain wall states (2) or Majorana fermion modes (19).

We now discuss the appearance of the peaks labeled E_-^* and E_+^* ($\sim \pm 10$ meV from E_{DP1}) in Fig. 3A. The immediate observation is that the peak energies do not change with magnetic field strength. This rules out g -factor effects (or a Zeeman term). Furthermore, the appearance of

Fig. 1. Crystal structure and schematic band structure of TCIs. (A) Crystal structure of $Pb_{1-x}Sn_xSe$. The top view reflects the [001] plane, which is the surface seen in STM. The Sn and Pb atoms are expected to randomly occupy the blue sites. (B) Schematic band structure of the surface state showing the surface Brillouin zone as a blue plane and the four pairs of Dirac nodes, placed across the $\bar{X}$ point in momentum space. (C) Typical dI/dV spectrum in zero field. (D) Cuts along two high-symmetry directions showing the calculated surface-state dispersion [calculated from our fit to the SM data (14)] of one of the four Dirac cones inside the BZ. The energy scales E_{DP1} , E_{VHS+} , E_{VHS-} , E_{DP2+} , and E_{DP2-} represent the Dirac point associated with the Dirac node deep in the band gap, the two Van Hove singularities associated with the saddle points in the dispersion, and the two higher-energy Dirac points associated with the Dirac nodes at the $\bar{X}$ point, close to bottom of the conduction band and the top of the valance band, respectively.

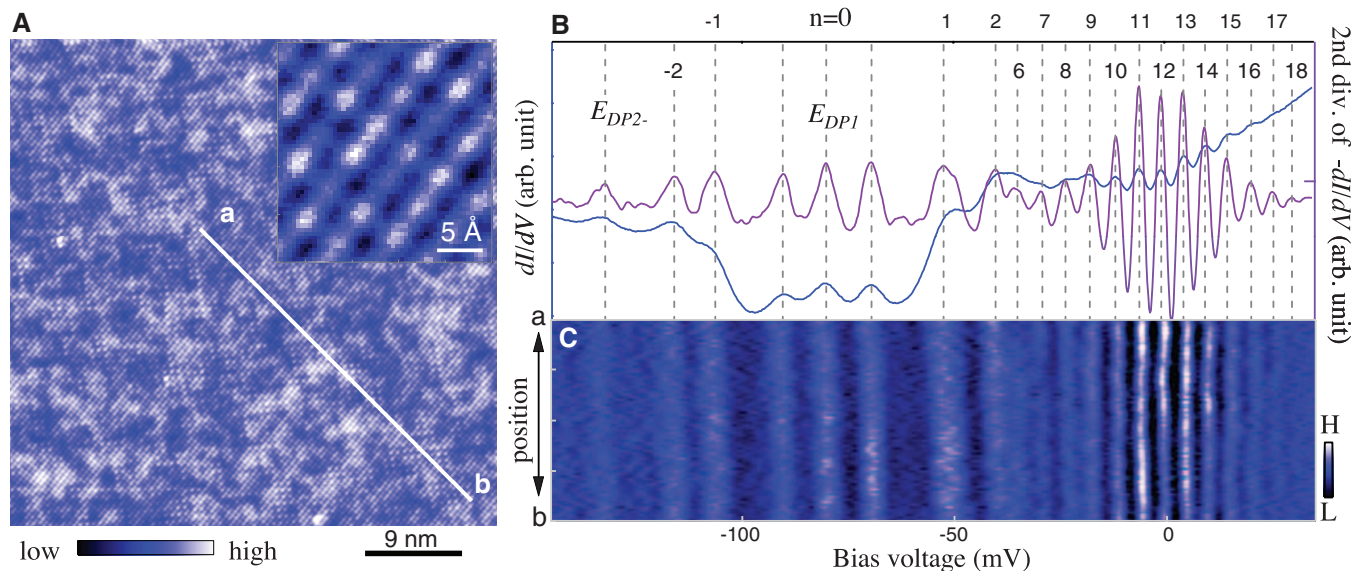
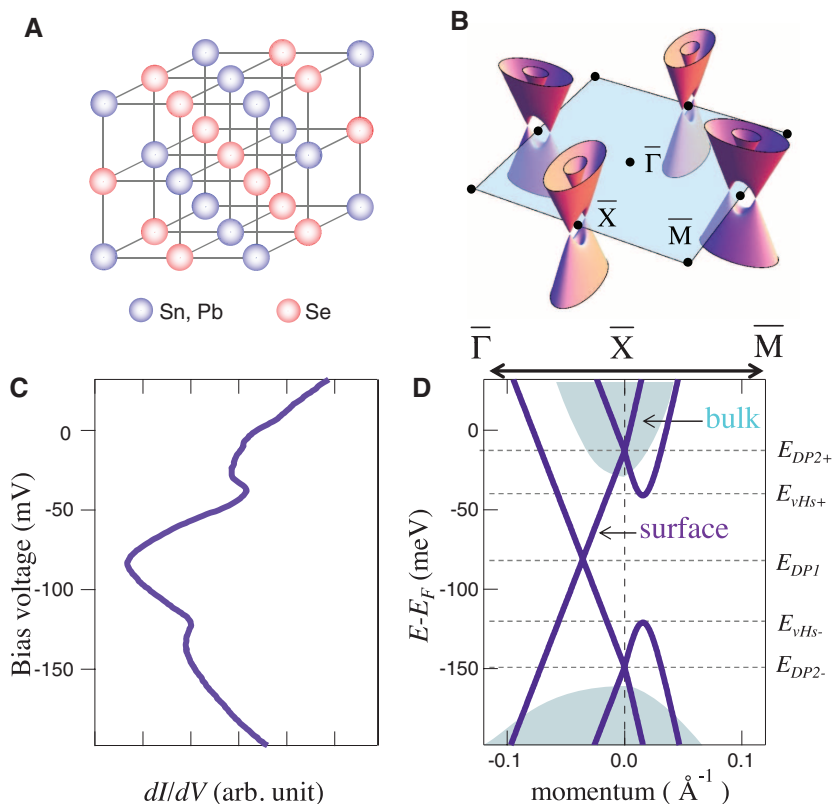
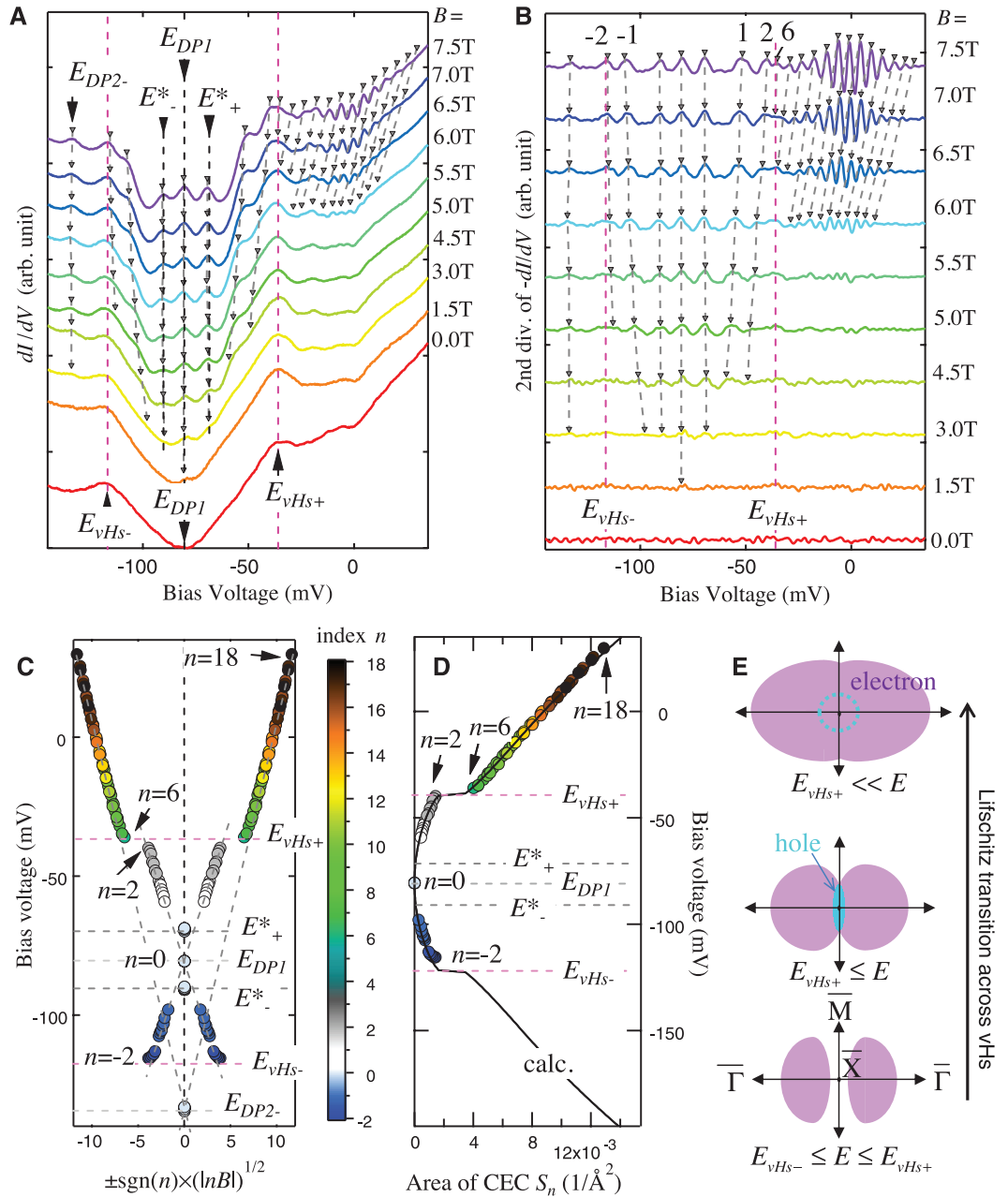


Fig. 2. STM image- and position-dependent spectra on $Pb_{1-x}Sn_xSe$. (A) Typical 40-nm STM topographic image at -100 -meV bias voltage with clearly resolved atoms as seen in the smaller-scale zoomed-in image in the inset. (B) Averaged STM spectrum at 7.5 T showing Landau levels (blue). The second derivative spectrum (purple) shows the LL peaks much

more clearly. Second derivative of spectra were therefore used to identify the peak positions. (C) Intensity plot of the second derivative LL spectra at 7.5 T as a function of position along the line shown in (A). The homogeneous nature of the sample is reflected in the lack of variation in the peak energy with position.

Fig. 3. Change in Fermi surface topology measured by Landau level spectroscopy. (A) Linecut averaged STM spectra at various values of magnetic field strength. (B) Second derivative of spectra shown in (A) that were used to trace the peak positions. (C) Plot of LL peak positions with $\sqrt{nB}$. The data here are from spectra at the 10 different magnetic fields shown in (B). The pink dotted lines in (A) and (B) indicate the energies of the VHS peaks, which persist in magnetic field. (D) Plot of theoretically calculated Fermi surface area with energy, overlaid with experimental LL peak positions [from spectra in (B)] as a function of nB [details are available in (14)]. The jump in area corresponds to the Lifschitz transition. (E) Schematic of evolution of the constant energy contours in momentum space with energy showing the Lifschitz transition and associated jump in Fermi surface area as we go from the bottom to the middle. The dotted circle at top indicates the inner surface-state band, which is expected to merge with the bulk conduction bands at higher energies.



the additional peaks is restricted to the vicinity of the Dirac point E_{DP1} , whereas all the other LL peaks (except E_{DP2-} , which we discuss later) can in principle be accounted for by the surface-state dispersion. If, however, the Dirac node (at E_{DP1}) is gapped out by the acquisition of a small mass term at zero-field, the resulting massive two-dimensional Dirac fermions will cause the appearance of a nondispersing $n = 0$ LL pinned at the energy of the Dirac mass (20). Mass acquisition could therefore potentially explain the existence of the E_-^* and E_+^* peaks. Their coexistence with the zero-mode LL peak at the gapless Dirac point E_{DP1} , however, places strong constraints on the origin of the Dirac mass. In TCIs, such a scenario can only be realized when mirror symmetry is broken

in one direction. Because there are two mirror planes within the surface BZ, it is possible to selectively break mirror symmetry reflection with respect to one mirror plane such as (110), leaving the other mirror plane unaffected. This can be achieved, for example, by a rhombohedral or orthorhombic distortion of the crystal structure with a displacement of atoms at the surface (Fig. 4), which is a common distortion in this class of materials (21–23). Because only one of the two sublattices was visible in our STM images (Fig. 2A), we could not directly image this distortion.

In order to confirm this scenario, we calculated the Landau level spectra theoretically by adding a new symmetry-breaking mass term to the recently developed $\mathbf{k} \cdot \mathbf{p}$ Hamiltonian for

TCIs (6). The resulting LL spectrum as a function of magnetic field is presented as a fan diagram in Fig. 4. Comparing the LL fan-diagram without (Fig. 4B) and with (Fig. 4C) the symmetry-breaking term, the mass term (with an appropriately chosen mass of ~ 11 meV) partially splits the fourfold degenerate 0th LL, whereas the LL spectra at higher energies are not substantially affected.

Comparing the theoretical model for coexisting massive and massless Dirac fermions to our experimental data, we found good agreement between the two. The symmetry-breaking term gaps out two of the four nodes, but the sign of the mass term for the two gapped nodes is necessarily opposite, as dictated by time-reversal symmetry (2). As a consequence, the $n = 0$ LLs for the two

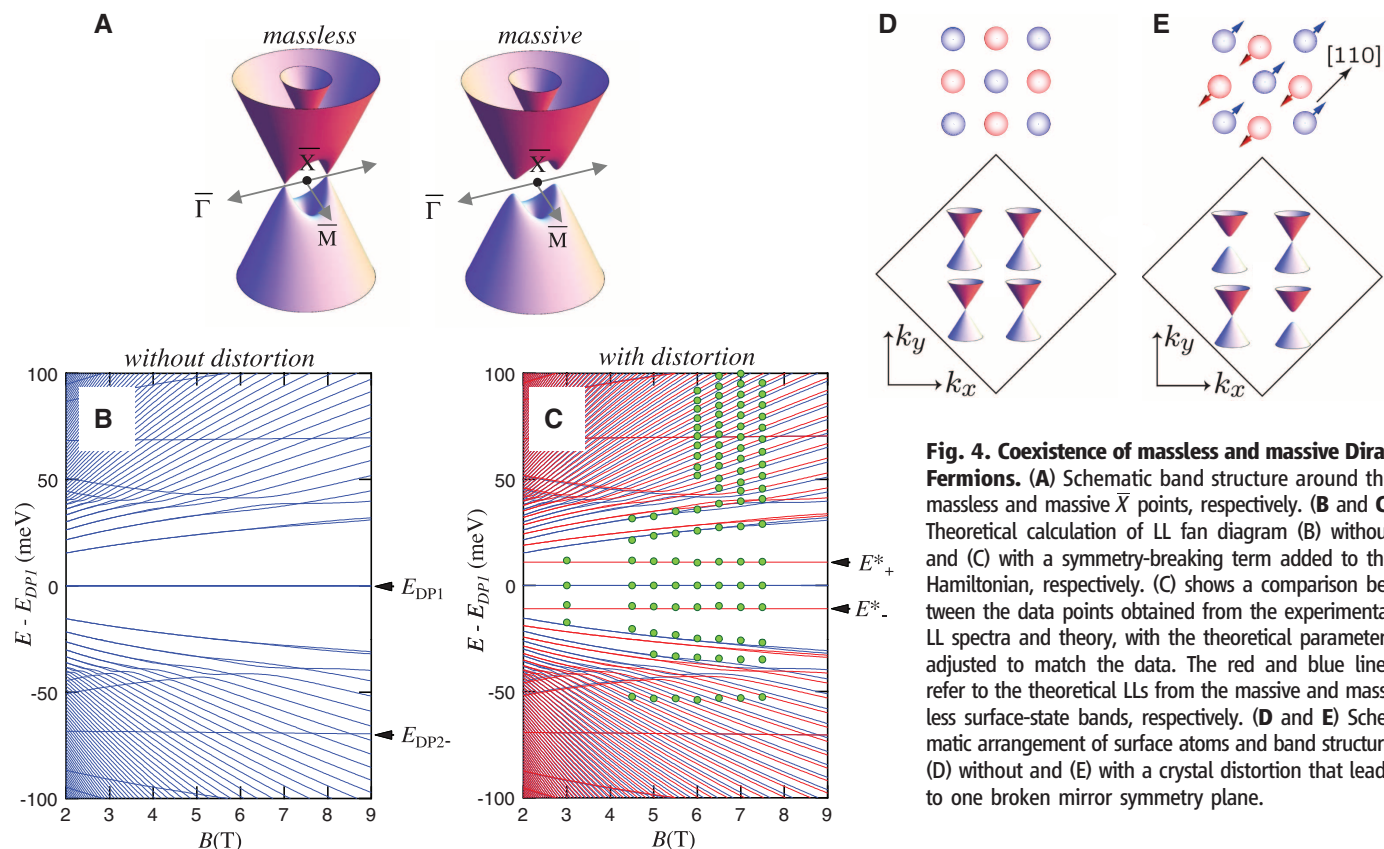


Fig. 4. Coexistence of massless and massive Dirac Fermions. (A) Schematic band structure around the massless and massive $\bar{X}$ points, respectively. (B and C) Theoretical calculation of LL fan diagram (B) without and (C) with a symmetry-breaking term added to the Hamiltonian, respectively. (C) shows a comparison between the data points obtained from the experimental LL spectra and theory, with the theoretical parameters adjusted to match the data. The red and blue lines refer to the theoretical LLs from the massive and massless surface-state bands, respectively. (D and E) Schematic arrangement of surface atoms and band structure (D) without and (E) with a crystal distortion that leads to one broken mirror symmetry plane.

massive Dirac bands appear on the upper and lower branch of the spectrum, respectively, each of which is nondegenerate. This explains our observation of two symmetric peaks near the Dirac point, E_{DP1} . All the other Landau levels are only weakly affected by the symmetry breaking.

By fitting the theoretical LLs to our STM data shown as green dots in Fig. 4C, excluding the peaks labeled E_{DP2-} , we can calculate the surface-state dispersion for the massive and massless cones (Fig. 1D and fig. S3). The theoretical saddle point energy is ~ 40 meV from the Dirac point E_{DP1} , which is once again consistent with our previous identification of the zero-field dI/dV peaks with VHSs. This analysis also provides a possible identification of the nondispersing feature labeled E_{DP2-} . Directly after the Dirac cones across the BZ merge, Dirac points appear at $\bar{X}$, as shown in Fig. 1D (E_{DP2+} and E_{DP2-}) and fig. S3 (14), which also result in nondispersing features in the LL calculations. Although this is suggestive that the experimental E_{DP2-} may originate from the Dirac point at $\bar{X}$, the theory and experimental energies are not identical. This could potentially be attributed to particle-hole asymmetry in the band structure, which is not included in the current model. The possibility of particle-hole asymmetry is also consistent with the observation that the LL data below E_{DP1} show deviations from the theoretical LL spectrum (Fig. 4C).

Our data show an enhanced, nearly singular density of states inside in the bulk band gap and tied to the surface-state spectrum near the Dirac

point. The band structure enabled by the coexistence of both massive and massless Dirac fermions in the same surface spectrum—as well as the enhanced density of states in close proximity to the Dirac point—demonstrates that $\text{Pb}_{1-x}\text{Sn}_x\text{Se}$ constitutes a realistic, tunable platform for exploring previously unknown topological states emergent via coupling to symmetry-breaking interactions.

References and Notes

1. L. Fu, *Phys. Rev. Lett.* **106**, 106802 (2011).
2. T. H. Hsieh *et al.*, *Nat. Commun.* **3**, 982 (2012).
3. Y. Tanaka *et al.*, *Nat. Phys.* **8**, 800–803 (2012).
4. S. Y. Xu *et al.*, *Nat. Commun.* **3**, 1192 (2012).
5. P. Dziawa *et al.*, *Nat. Mater.* **11**, 1023–1027 (2012).
6. J. Liu, W. Duan, L. Fu, <http://arxiv.org/abs/1304.0430>.
7. I. M. Lifshitz, *Sov. Phys. JETP* **11**, 1130 (1960).
8. R. S. Markiewicz, *J. Phys. Condens. Matter* **2**, 665–676 (1990).
9. W. Kohn, J. M. Luttinger, *Phys. Rev. Lett.* **15**, 524–526 (1965).
10. T. M. Rice, G. K. Scott, *Phys. Rev. Lett.* **35**, 120–123 (1975).
11. M. Fleck, A. M. Oleś, L. Hedin, *Phys. Rev. B* **56**, 3159–3166 (1997).
12. J. González, *Phys. Rev. B* **78**, 205431 (2008).
13. R. Nandkishore, L. Levitov, A. Chubukov, *Nat. Phys.* **8**, 158–163 (2012).
14. Materials and methods are available as supplementary materials on Science Online.
15. Y. Okada *et al.*, *Phys. Rev. Lett.* **109**, 166407 (2012).
16. G. Li, Y. Eva, *Nat. Phys.* **3**, 623–627 (2007).
17. T. Hanaguri, K. Igarashi, M. Kawamura, H. Tagaki, T. Sasagawa, *Phys. Rev. B* **82**, 081305 (2010).
18. G. Li *et al.*, *Nat. Phys.* **6**, 109–113 (2010).
19. F. Zhang, C. L. Kane, E. J. Mele, <http://arxiv.org/abs/1303.4144>.
20. F. D. M. Haldane, *Phys. Rev. Lett.* **61**, 2015–2018 (1988).
21. K. Adouby, C. Perez-Vicente, J. C. Jumas, R. Fourcade, A. Abba Toure, *Z. Kristallogr.* **213**, 343–349 (1998).
22. M. Iizumi, Y. Hamaguchi, K. F. Komatsubara, Y. Kato, *J. Phys. Soc. Jpn.* **38**, 443 (1975).
23. M. Snykers, P. Delavignette, S. Amelinckx, *Mater. Res. Bull.* **7**, 831–839 (1972).

Acknowledgments: V.M. gratefully acknowledges funding from the U.S. Department of Energy (DOE), Scanned Probe Division under award DE-FG02-12ER46880 for the support of Y.O. and D.W. acknowledges NSFDMR-1056625 for support of C.D. The work at Northeastern University is supported by the DOE Office of Science, Basic Energy Sciences contract DE-FG0207ER46352 and benefited from Northeastern University's Advanced Scientific Computation Center. L.F. is partly supported by the DOE Office of Basic Energy Sciences, Division of Materials Sciences and Engineering under award DE-SC0010526. M.S. was supported by P. A. Lee via grant NSF DMR 1104498. The work at Princeton and Princeton-led synchrotron x-ray-based measurements (angle-resolved photoemission spectroscopy) are supported by the DOE Office of Basic Energy Sciences, grant DE-FG-02-05ER46200. M.Z.H. acknowledges visiting-scientist support from the Lawrence Berkeley National Laboratory and support from the A. P. Sloan Foundation. We thank Y. Ran and I. Zeljkovic for useful discussions.

Supplementary Materials

www.sciencemag.org/content/341/6153/1496/suppl/DC1
Materials and Methods
SupplementaryText
Figs. S1 to S4
Reference (24)

22 April 2013; accepted 22 August 2013
Published online 29 August 2013;
10.1126/science.1239451

Abnormal Grain Growth Induced by Cyclic Heat Treatment

Toshihiro Omori,^{1*} Tomoe Kusama,¹ Shingo Kawata,¹ Ikuro Ohnuma,¹ Yuji Sutou,¹ Yoshikazu Araki,² Kiyohito Ishida,¹ Ryosuke Kainuma¹

In polycrystalline materials, grain growth occurs at elevated temperatures to reduce the total area of grain boundaries with high energy. The grain growth rate usually slows down with annealing time, making it hard to obtain grains larger than a millimeter in size. We report a crystal growth method that employs only a cyclic heat treatment to obtain a single crystal of more than several centimeters in a copper-based shape-memory alloy. This abnormal grain growth phenomenon results from the formation of a subgrain structure introduced through phase transformation. These findings provide a method of fabricating a single-crystal or large-grain structure important for shape-memory properties, magnetic properties, and creep properties, among others.

Most metals and ceramics have a polycrystalline structure that is composed of single-crystalline grains separated by interfaces, called grain boundaries (GBs). There are multiple types of GBs—including low-angle, high-angle, and coincidence—that each possess a partial randomness in the position of the atoms and are of higher energy than the neighboring crystal. Therefore, the polycrystalline structure is thermodynamically unstable, and the average grain size can increase during heat treatment to reduce the total GB area. The increase of average grain size occurs due to a gradual growth of the larger grains and elimination of the smaller ones, through GB migration. In certain circumstances, selective growth of a few grains occurs by engulfing the neighboring ones, resulting in formation of considerably huge grains; this phenomenon is known as abnormal grain growth (AGG). AGG is used in the fabrication process for Fe-Si electrical steels that have excellent magnetic properties with small core loss and are used in electrical power transformers, motors, and generators. In this alloy, the texture of Goss orientation ($\{110\}<001>$) showing higher magnetic property is realized by AGG (1) induced by second-phase (inhibitor) dispersion and thermomechanical treatment (2). Although the mechanism for AGG has not fully been understood, it has been reported that strong retardation of normal grain growth (3–5) is one of the important factors (2).

A well-known technique to obtain AGG is the strain-anneal method, consisting of a small macroscopic deformation followed by thermal annealing (6). AGG is brought about by encroaching subgrain structure, which is formed by rearrangement of dislocations introduced by the macroscopic deformation. It has also been demonstrated that a single crystal can be obtained in a wire and plate by straining and sub-

sequent annealing with a temperature gradient (7–9). Another technique to produce a large grain or a single crystal in the solid state is the dynamic AGG, consisting of plastic straining at elevated temperatures (10). These methods are only applicable to samples with a simple shape, such as a sheet or wire, in which fracture does not occur by slight plastic deformation.

We have determined a simpler method to obtain AGG by cyclic heat treatment without macroscopic deformation in Cu-Al-Mn shape-memory alloys (SMAs). Cu-Al-Mn (17 atomic % Al) alloys quenched from the β (body-centered cubic) single-phase region at high temperatures exhibit shape-memory properties associated with the martensitic phase transformation (11, 12). According to the Cu-Al-Mn (10 atomic % Mn) phase diagram (fig. S1), because the Cu-Al-Mn (17 atomic % Al) alloy has the α (face-centered cubic) + β two-phase structure in the temperature region below $\sim 650^\circ\text{C}$, the precipitation of the α phase occurs during cooling from the β -phase region or isothermal aging at lower temperatures. AGG of the β phase was realized by a process (Fig. 1) of slow cooling from the β -phase region (e.g., 900°C) to the α + β two-phase region (e.g., 500°C) and subsequent heating to the β single-phase region (e.g., 800°C), as shown in Fig. 2B. Figure 2C shows the microstructure of a $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ alloy sheet obtained by the cyclic heat treatment illustrated in Fig. 1. Abnormally large grains with lengths from 5 to 22 mm are formed. In contrast, in an isothermally annealed sheet, the maximum grain diameter of the β phase is ~ 2 mm, as shown in Fig. 2A, even though the final annealing at 900°C was performed for a full 24 hours. Furthermore, AGG repeatedly occurs by multiple cyclic heat treatments, in contrast to the conventional AGG where the growth rate of abnormal grains drastically decreases with increasing mean grain size. Figure 2D shows the microstructure of the sheet specimen after five cyclic heat treatments. A grain approaching 50 mm in length was obtained with a total treatment time of 20 hours.

To understand the origin of the AGG, we used the electron backscatter diffraction (EBSD)

technique to analyze the microstructure in the initial stage of the grain growth. In Figure 3, panels A and B, respectively, show the quasi-colored orientation mapping of the β phase and the grain reference orientation deviation indicating the deviation from the average orientation of the grain for $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ quenched from 730°C , which is just above the α solvus temperature (726°C). Surprisingly, small subgrains with a mean diameter of ~ 100 μm , possessing orientation deviations from 1° to 2° to one another, are observed in every preexisting grain. A similar subgrain structure is formed by recovery through deformation and low-temperature annealing. The subgrains, however, are consumed by recrystallization through further high-temperature annealing. Thus, the fully recrystallized specimen contains only a low density of dislocations and subboundaries in the recrystallized grains. In the present case, because the specimens were initially annealed at a high enough temperature to complete the recrystallization process, this subgrain structure is not attributed to the strain annealing. No subgrain structure was observed after water quenching from the β single-phase region without cyclic heat treatment, meaning that the subgrains were not produced during water quenching but were preserved from the high-temperature state. The formation of such a subgrain structure occurs in the precipitation and dissolution of the α phase during the cyclic heat treatment after recrystallization. Although the mechanism responsible for the AGG has not yet been determined, the formation of the subgrain structure is strongly related to this phenomenon. For example, the subgrain-boundary energy might be one of the driving forces.

To clarify the formation mechanism of the subgrain structure, we used EBSD to examine the two-phase microstructure after precipitation of the α phase. Figure 4A shows the quasi-colored orientation mapping of the β phase (top) and α phase (middle) and grain reference orientation deviation mapping of the β phase (bottom) in a Cu-Al-Mn alloy slowly cooled from 900° to 500°C . In the grain reference orientation deviation map, the orientation gradients up to 3° and, occasionally, 5° are visible in the β phase around the α precipitates. Figure 4B shows the

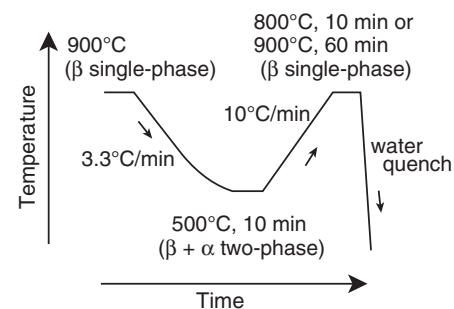


Fig. 1. Overview of the annealing processes. Thermally cycling the β phase leads to a precipitation and subsequent dissolution of the α phase.

¹Department of Materials Science, Graduate School of Engineering, Tohoku University, 6-6-02 Aoba-yama, Sendai 980-8579, Japan. ²Department of Architecture and Architectural Engineering, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo, Kyoto 615-8540, Japan.

*Corresponding author. E-mail: omori@material.tohoku.ac.jp

Fig. 2. The microstructure of a Cu-Al-Mn alloy with simple isothermal heat treatment and cyclic heat treatment. (A) The microstructure of a $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ alloy sheet annealed at 900°C for 24 hours. (B) The microstructure of the $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ alloy annealed at 900°C for 5 min, followed by cooling to 500°C and, subsequently, heating to 800°C with a holding time of 10 min. One grain abnormally grows to ~7 mm. (C) The microstructure of a $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ alloy sheet annealed at 900°C for 10 min, followed by cooling to 500°C and subsequent heating to 900°C with a holding time of 60 min. (D) The microstructure of a $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ alloy sheet subjected to five cycles of the above-mentioned heat treatment, showing grains with sizes on the centimeter scale.

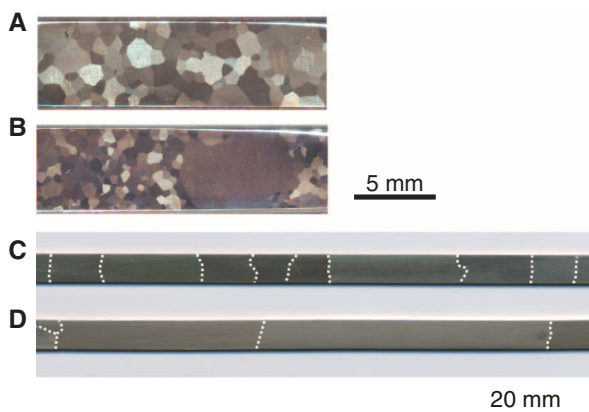


Fig. 3. Crystallographic orientation of a Cu-Al-Mn sheet heated to 730°C and then quenched. (A) Quasi-colored orientation mapping (OM) of a $\text{Cu}_{71.6}\text{Al}_{17}\text{Mn}_{11.4}$ alloy sheet quenched from 730°C in the heating process after cooling from 900° to 500°C. The EBSD technique was used to analyze the microstructure. The colors correspond to crystal direction parallel to the normal direction given in the stereographic triangle. (B) Grain reference orientation deviation (GROD) mapping, where the color value of every pixel is calculated as the misorientation angle of this pixel with respect to a reference orientation (average orientation of a grain) in the same grain. Subgrains are shown in every normal grain. Arrows indicate the migration of GBs.

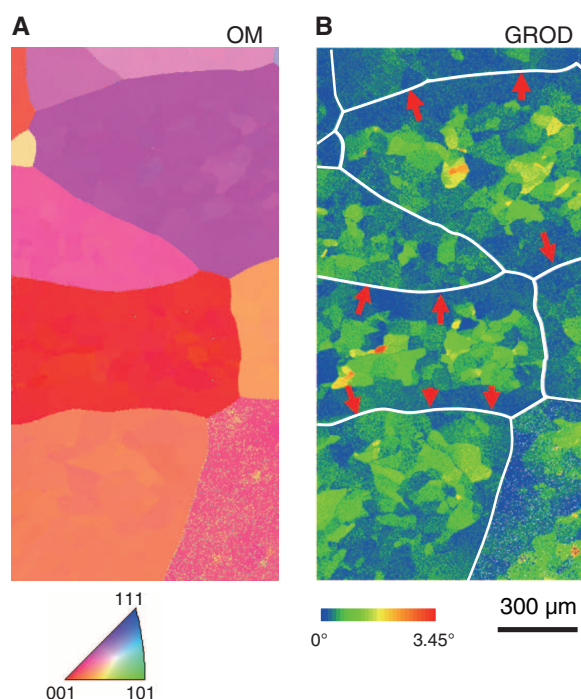
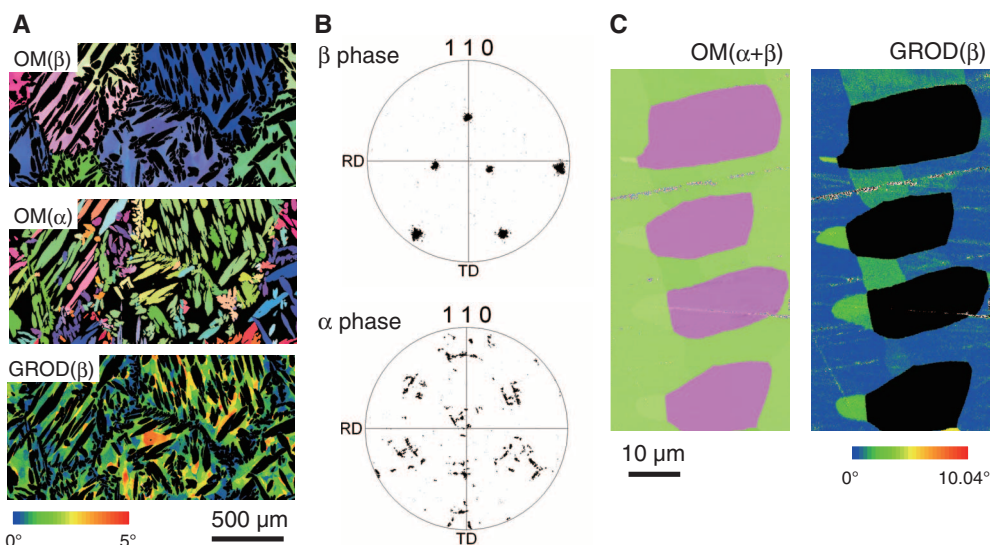


Fig. 4. Microstructure and crystallographic orientation in the α and β two-phase of a Cu-Al-Mn alloy cooled from 900°C. (A) Quasi-colored OM of the β phase (top) and the α phase (middle) in normal direction and GROD mapping of the β phase (bottom) of Cu-Al-Mn quenched from 500°C. (B) (110) pole figure of the β phase (top) and α phase (bottom) of Cu-Al-Mn quenched from 500°C. RD, rolling direction; TD, transverse direction. (C) Quasi-colored OM of the α and β phases (left) in normal direction and GROD mapping of the β phase (right) of a Cu-Al-Mn alloy quenched from 680°C, showing the formation of a subgrain structure in the matrix phase. The color key for OMs is given in the stereographic triangle of Fig. 3A.



(110) pole figures of the β and α phases in the Cu-Al-Mn alloy cooled to 500°C. Each β grain contains a broad orientation, which is due to the orientation variation by the precipitation of the α phase, and the almost-isotropic broadening means that the orientation changes in many different directions. The orientation relationship between β and α phases is shown in Fig. 4B, where the Bain orientation relationship ($\{010\}_{\beta} // \{010\}_{\alpha}$, $\langle 101 \rangle_{\beta} // \langle 001 \rangle_{\alpha}$), K-S orientation relationship ($\{110\}_{\beta} // \{111\}_{\alpha}$, $\langle 1\bar{1}1 \rangle_{\beta} // \langle 1\bar{1}0 \rangle_{\alpha}$), and Pitsch orientation relationship ($\{110\}_{\beta} // \{100\}_{\alpha}$, $\langle \bar{1}11 \rangle_{\beta} // \langle 011 \rangle_{\alpha}$) are detected in several observed areas. A typical microstructure including the coarse and large precipitates observed for the specimen slowly cooled to 680°C is shown in Fig. 4C. The subgrain boundary apparently expands from the interphase boundary to the β -phase matrix region, and the orientation change in the β grains becomes visible. The interface between the α and β phases is semicoherent (fig. S2), and the orientation gradient of the β matrix is more noticeable around large α precipitates than that around fine precipitates. This result suggests that the subgrain boundary may be formed through loss of coherency of the growing precipitate, generating dislocations in the matrix (13–15). This discussion could be understood in relation to the internal stress superplasticity induced by thermal cycling under an external stress, where internal stress arises from the differences in volume or thermal expansion coefficient between two phases, etc. (16–25). Such subgrains introduced in the β matrix clearly remain, even after dissolution of the α phase during heating to the β single-phase region, and the fully subgrained structure is finally formed. Because the subgrain structure is easily formed by a cyclic heat treatment, AGG can be repeatedly induced with no macroscopic deformation. Therefore, it may be possible to apply this technique to other alloys that form semicoherent precipitates at lower temperatures.

From the viewpoint of material cost, cold workability, and machinability, Cu-based SMAs are superior to the commonly used Ti-Ni alloy (11, 12, 26). In Cu-Al-Mn alloys, the stress-strain behavior of polycrystalline alloys strongly depends on their grain size relative to the diameter of wires or to the thickness and width of sheets (12, 27, 28). When the mean grain size is sufficiently smaller than the cross-sectional sizes, three-dimensionally constrained grains cause large constraint during deformation due to the incompatibility of transformation strain at GBs, resulting in deterioration of superelasticity due to plastic deformation. In a bamboo structure, in which grains transverse the cross section as shown in Fig. 2, C and D, the grain constraint is drastically decreased, and Cu-Al-Mn alloys can show high superelastic strain comparable to Ti-Ni alloys. This feature has limited the use of this alloy to thin wires (<1.5 mm in diameter) (12, 27) or thin sheets, because the formation of a bamboo structure through the normal grain growth is not easy. We have successfully obtained two Cu-Al-Mn alloy bars with 15- and 30-mm diameters with a bamboo structure using the AGG method, and we have confirmed excellent superelasticity (fig. S3). The superelastic properties of Cu-Al-Mn alloys have potential for seismic applications (26).

References and Notes

- N. P. Goss, *Trans. ASM* **23**, 511–531 (1934).
- F. J. Humphreys, M. Hatherly, in *Recrystallization and Related Annealing Phenomena* (Elsevier, Oxford, ed. 2, 2004), pp. 368–378.
- C. S. Smith, *Trans. AIME* **175**, 15–51 (1948).
- W. Mullins, *Acta Metall.* **6**, 414–427 (1958).
- E. A. Holm, S. M. Foiles, *Science* **328**, 1138–1141 (2010).
- H. C. H. Carpenter, C. F. Elam, *Proc. R. Soc. A* **100**, 329–353 (1921).
- T. Fujiwara, T. Hudita, *J. Sci. Hiroshima Univ. Ser. A* **8**, 293–296 (1938).
- T. Fujiwara, *J. Sci. Hiroshima Univ. Ser. A* **9**, 227–231 (1939).
- T. Fujiwara, K. Yamasaki, *J. Sci. Hiroshima Univ. Ser. A* **11**, 89–92 (1941).
- J. Ciolik, E. M. Taleff, *Scr. Mater.* **61**, 895–898 (2009).
- R. Kainuma, S. Takahashi, K. Ishida, *Metall. Mater. Trans. A* **27A**, 2187–2195 (1996).
- Y. Soutou, T. Omori, R. Kainuma, K. Ishida, *Mater. Sci. Technol.* **24**, 896–901 (2008).
- J. W. Martin, R. D. Doherty, B. Cantor, in *Stability of Microstructure in Metallic Systems* (Cambridge Univ. Press, Cambridge, ed. 2, 1997), pp. 28–83.
- G. C. Weatherly, *Philos. Mag.* **17**, 791–799 (1968).
- L. M. Brown, G. R. Woolhouse, *Philos. Mag.* **21**, 329–345 (1970).
- O. D. Sherby, J. Wadsworth, *Prog. Mater. Sci.* **33**, 169–221 (1989).
- P. Zwigl, D. C. Dunand, *Metall. Mater. Trans. A* **29A**, 2571–2582 (1998).
- Q. Li, E. Y. Chen, D. R. Bice, D. C. Dunand, *Metall. Mater. Trans. A* **38A**, 44 (1998).
- C. Schuh, P. Noël, D. C. Dunand, *Acta Mater.* **48**, 1639–1653 (2000).
- C. Schuh, D. C. Dunand, *Acta Mater.* **49**, 199–210 (2001).
- B. Ye, M. R. Matsen, D. C. Dunand, *Acta Mater.* **58**, 3851–3859 (2010).
- P. Zwigl, D. C. Dunand, *Acta Mater.* **45**, 5285–5294 (1997).
- M. Y. Wu, J. Wadsworth, O. D. Sherby, *Metall. Trans. A* **18A**, 451–462 (1987).
- G. González-Doncel, O. D. Sherby, *Metall. Mater. Trans. A* **27A**, 2837–2842 (1996).
- C. Schuh, D. C. Dunand, *Acta Mater.* **49**, 3387–3400 (2001).
- Y. Araki *et al.*, *Earthquake Eng. Struct. Dynam.* **40**, 107–115 (2011).
- Y. Soutou *et al.*, *Acta Mater.* **53**, 4121–4133 (2005).
- Y. Soutou, T. Omori, R. Kainuma, K. Ishida, *Acta Mater.* **61**, 3842–3850 (2013).

Acknowledgments: This work was supported by the Japan Science and Technology Agency and by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and a Grant for Excellent Graduate Schools from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We thank K. R. A. Ziebeck, Cavendish Laboratory, University of Cambridge, for his help in critical reading of the manuscript. T.O., T.K., R.K., K.I., T. Tanaka, S. Kise, K. Nakamizo, K. Ishikawa, M. Nakano, and S. Teshigawara are inventors on Japanese patent application number 2013-099996, applied for by Tohoku University, Furukawa Techno Material Co., and Furukawa Electric Co.

Supplementary Materials

www.sciencemag.org/content/341/6153/1500/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S3

References (29–35)

19 March 2013; accepted 1 August 2013

10.1126/science.1238017

Cation Intercalation and High Volumetric Capacitance of Two-Dimensional Titanium Carbide

Maria R. Lukatskaya,^{1,2} Olha Mashtalir,^{1,2*} Chang E. Ren,^{1,2*} Yohan Dall'Agnese,^{1,2,3,4} Patrick Rozier,³ Pierre Louis Taberna,³ Michael Naguib,^{1,2} Patrice Simon,^{3,4} Michel W. Barsoum,¹ Yury Gogotsi^{1,2†}

The intercalation of ions into layered compounds has long been exploited in energy storage devices such as batteries and electrochemical capacitors. However, few host materials are known for ions much larger than lithium. We demonstrate the spontaneous intercalation of cations from aqueous salt solutions between two-dimensional (2D) Ti₃C₂ MXene layers. MXenes combine 2D conductive carbide layers with a hydrophilic, primarily hydroxyl-terminated surface. A variety of cations, including Na⁺, K⁺, NH₄⁺, Mg²⁺, and Al³⁺, can also be intercalated electrochemically, offering capacitance in excess of 300 farads per cubic centimeter (much higher than that of porous carbons). This study provides a basis for exploring a large family of 2D carbides and carbonitrides in electrochemical energy storage applications using single- and multivalent ions.

With the increased demand for portable and clean energy, electrochemical capacitors have been attracting attention because of their much greater power density and

cyclability relative to Li batteries (1, 2). However, electrical double-layer capacitors (EDLCs), in which the capacity is due to the electrosorption of ions on porous carbon electrodes, have limited energy density (2). Pseudo-capacitors, in which the capacity is due to redox reactions, provide higher energy densities but usually suffer from shorter cyclic lifetimes. RuO₂ nanosheets have been used in redox capacitors and have shown impressive capacitance and cyclability, but they are quite expensive to produce (2, 3).

Energy density enhancement of capacitors can be achieved by using hybrid devices, which

combine a battery-like redox electrode and a porous carbon electrode (4). Another approach is to use materials in which charge storage is due to intercalation of ions between atomic layers because the capacitances—even at high discharge rates—are high. For example, nanocrystalline Nb₂O₅ films with storage capacities of ~130 mAh g⁻¹ at rates as high as 10 C (charge/discharge in 6 min) for Li⁺ ions in organic electrolytes have been reported. The specific structure of this material can best be described as a crystalline network with two-dimensional (2D) transport paths for ions between atomic layers; thus, even thick electrodes show excellent behavior (5). Another example is Mg-buserite electrodes, which exhibit good Na⁺ ion intercalation capacitances but have poor electrical conductivities (6). Most materials for electrodes that can provide intercalation or surface redox capacitances are poor electronic conductors [e.g., graphene oxide or TiO₂ (7)] or are hydrophobic [e.g., graphene (8)].

Recently, we reported on a large family of 2D materials that we labeled “MXenes,” which combine good electrical conductivities with hydrophilic surfaces. MXenes are 2D materials synthesized by the extraction of the “A” layers from the layered carbides or carbonitrides known as MAX phases. The latter have a general formula of M_{n+1}AX_n (n = 1, 2, 3), where M represents a transition metal; A usually represents a III A or IV A element (such as Al, Ga, Si, or Ge); and X represents C and/or N (9). The MXenes Ti₃C₂ (10), Ti₂C, Ta₄C₃, TiNbC, and (V_{0.5}Cr_{0.5})₃C₂ (11), have been fabricated by immersing Al-containing MAX powders in HF solution at room or slightly

¹Department of Materials Science and Engineering, Drexel University, Philadelphia, PA 19104, USA. ²A. J. Drexel Nanotechnology Institute, Drexel University, Philadelphia, PA 19104, USA.

³Université Paul Sabatier, CIRIMAT UMR CNRS 5085, 118 route de Narbonne, 31062 Toulouse, France. ⁴Réseau sur le Stockage Electrochimique de l'Energie (RS2E), FR CNRS 3459, France.

*These authors contributed equally to this work.

†Corresponding author. E-mail: gogotsi@drexel.edu

elevated temperature (12). This synthesis procedure leads to the termination of MXene surfaces by primarily O and/or OH groups with some fluorine present. These terminated MXenes are of the form $M_{n+1}X_nT_x$, where T represents surface termination (O, OH, and/or F) and x is the number of termination groups. We recently showed that $Ti_3C_2T_x$ can be readily and spontaneously intercalated with organic molecules such as hydrazine, dimethyl sulfoxide (DMSO), and urea (13).

MXenes have shown promise as electrode materials for Li-ion batteries and Li-ion capacitors (13, 14). For example, when Li^+ ions intercalate into $Ti_3C_2T_x$, a steady-state capacity of $\sim 410 \text{ mAh g}^{-1}$ at 1 C is obtained for additive-free flexible electrodes (13). Furthermore, theoretical calculations have predicted that Li^+ ions should diffuse rapidly on Ti_3C_2 surfaces, as well as result in high storage capacities (15). However, spontaneous intercalation of cations from aqueous solutions has neither been theoretically predicted nor experimentally demonstrated.

Here, we report on the intercalation of Li^+ , Na^+ , Mg^{2+} , K^+ , NH_4^+ , and Al^{3+} ions between the 2D $Ti_3C_2T_x$ layers (Fig. 1A). In most cases, the cations intercalated spontaneously. A schematic of the process is shown in Fig. 1B. The intercalation of some ions, notably Al^{3+} , can additionally be promoted electrochemically. We also report on intercalation-induced high capacitances of flexible $Ti_3C_2T_x$ paper electrodes in aqueous electrolytes.

A large number of salts, bases, and acids were explored (table S1). X-ray diffraction (XRD) patterns showed that, after placing the $Ti_3C_2T_x$ in various salt solutions (fig. S1, A to C), there was a downshift in the (0002) peak position. This downshift shows that in all cases, there was an increase in the c -lattice parameter. For example, the c value of $Ti_3C_2T_x$ increased from 20.3 Å to as much as 25.4 Å when placed in potassium hydroxide (KOH) and ammonium hydroxide (NH_4OH) solutions (fig. S1A). In addition to the compounds listed in fig. S1, A to C, other salts intercalated spontaneously when the MXene powders were immersed in sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), or lithium hydroxide (LiOH) solutions.

Not all salts behaved similarly. In the case of high-pH solutions such as KOH, NH_4OH , NaOH, LiOH, and several others (table S1), the changes in the interplanar spacing were large (fig. S1A). Conversely, close-to-neutral solutions such as Na,

K, and Mg sulfates resulted in smaller changes in c (fig. S1B; see also table S1). No shift in the (0002) peak positions was observed when $Ti_3C_2T_x$ was immersed in acetic or sulfuric acid.

To shed light on whether the cations or anions intercalated the $Ti_3C_2T_x$ layers, we tested three sodium salts with differing anion radii. The results (fig. S1C) showed that the c -axis expansions were comparable and independent of anion radii. Furthermore, energy-dispersive x-ray spectroscopy analysis of $Ti_3C_2T_x$ after treatment in the different sulfate salts (fig. S1B) confirmed the presence of the cations; sulfur was not detected (table S2), confirming that it is the cations that intercalate between the $Ti_3C_2T_x$ layers.

Materials with large specific surface area are typically needed to obtain large capacitances in carbon materials for EDLCs. However, at $23 \text{ m}^2/\text{g}$, the surface area of multilayer exfoliated $Ti_3C_2T_x$ is low (13). It follows that if double-layer capacitance were the only operative mechanism, one would expect the capacitance for this material to be less than that of (for example) activated graphene by a factor of 100 (16). However, as noted above, intercalation capacitance can by far exceed double-layer capacitances calculated solely on the basis of a material's surface area (6, 17).

To test this idea, we fabricated multilayer $Ti_3C_2T_x$ electrodes (see supplementary materials for details) and tested them in NaOH-, KOH-, and LiOH-containing electrolytes using a standard three-electrode asymmetrical setup with an Ag/AgCl reference electrode (fig. S2). The resulting cyclic voltammograms (CVs) are shown in Fig. 2A [see fig. S4 for the corresponding electrochemical impedance spectroscopy (EIS) results]. The rectangular-shaped CVs indicate capacitive behavior in these basic solutions. Note that in all experiments, the open circuit potential (OCP) was taken as the starting potential for the CV scans because 0.1 V above this potential, $Ti_3C_2T_x$ oxidation is observed in aqueous electrolytes (see fig. S3).

To study the effect of a cation's valence on the electrochemical performance of multilayer exfoliated $Ti_3C_2T_x$ electrodes, we performed CV scans in 1 M solutions of potassium and aluminum sulfates and nitrates (Fig. 2B and fig. S4B). Clearly, the responses in the K^+ - and Al^{3+} -containing solutions were distinctively different, confirming once again that the cations (and not the anions) are intercalating. The CV plots for K_2SO_4 are almost perfectly rectangular. Conversely, the CV

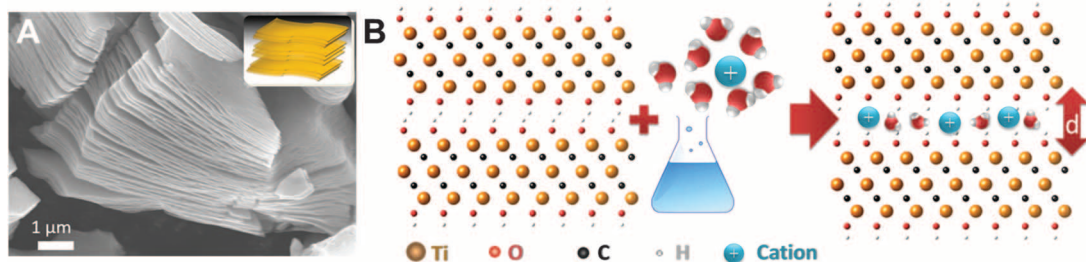
data for the more acidic (see table S3) and less conductive $Al_2(SO_4)_3$ electrolyte yield capacitance values that are significantly lower, and the shape of the CV at 10 mV/s and the EIS results show a higher resistance (Fig. 2B and fig. S4B). To ensure that lower electrolyte conductivity did not limit the capacitive performance, we tested $Ti_3C_2T_x$ in 1 M $Al(NO_3)_3$, which has a conductivity similar to that of 1 M K_2SO_4 (table S3). Although the normalized capacitance did not increase appreciably, the CV loops were definitely more rectangular (Fig. 2B), demonstrating the role of electrolyte conductivity.

Further evidence for cation intercalation and its beneficial effect on capacitance comes from the observation that for some electrolytes, time was needed to reach a steady state or maximum capacitance. For strongly basic electrolytes (table S1), such as KOH solutions, the rectangular CV plots were observed almost immediately and the capacitances did not change with time or cycle number. For other electrolytes, however, there was a slow and gradual increase in capacitance with time. For example, for salts such as $MgSO_4$, the CV area increased steadily with time and the maximum capacity was reached only after 48 hours (see figs. S5 and S6). Unlike what is observed for graphite (18), there was no irreversible capacitance loss during the first cycle for any of the electrolytes studied.

The performance of the multilayer $Ti_3C_2T_x$ in all tested electrolytes is summarized in Fig. 2C. The specific capacitances were calculated by integrating the discharge portions of the CV plots. The results clearly showed responses that depended on the electrolytes used. Moreover, the calculated capacitances were quite high for a material with such low surface area.

In situ XRD studies of the intercalation process during cycling showed that electrochemical cycling leads to insignificant changes in the c values. For example, when a $Ti_3C_2T_x$ electrode was cycled in a KOH-containing electrolyte, the c values fluctuated within 0.33 Å as the potential was scanned from -1 to -0.2 V (Fig. 3A). Interestingly, a slight shrinkage in c values was observed with increasing voltage. Similar behavior was observed when $Ti_3C_2T_x$ was cycled in NaOAc-containing electrolyte (fig. S7). The simplest explanation for this observation is that the positively charged ions incorporated in $Ti_3C_2T_x$ increase the electrostatic attraction between layers, in a manner analogous to what is observed for

Fig. 1. Intercalation of multilayer exfoliated $Ti_3C_2T_x$. (A) Scanning electron micrograph of the $Ti_3C_2T_x$ layered particle. Inset is a schematic of the same, showing the 2D nature of MXenes. (B) Schematic illustration of the intercalation of cations between $Ti_3C_2T_x$ layers. The interlayer spacing d increases after ion intercalation.



MnO₂ (19). When Ti₃C₂T_x was electrochemically cycled in a MgSO₄-containing solution, the shift of the (0002) peak almost doubled relative to the KOH and NaOAc electrolytes (compare Fig. 3A and Fig. 3B). Here again, a slight shrinkage in *c* values was observed with increasing voltage.

To gain further insight into the capacitances and what influences them, we tested MXene “paper” produced by filtering delaminated Ti₃C₂T_x (Fig. 4B). This paper, with a specific surface area of 98 m²/g, is flexible (inset in Fig. 4B), hydrophilic, additive-free, and conductive. When tested in KOH, the CVs were rectangular, similar to those obtained when multilayer Ti₃C₂T_x powder was used (compare Fig. 4C to Fig. 2A). Furthermore, the EIS results indicated that the Ti₃C₂T_x paper-based capacitors were less resistive (Fig. 4D) than those made with multilayer Ti₃C₂T_x (fig. S4A). This improved electrochemical response can be related to a number of factors, such as the absence of a binder in the system, good contact between the restacked flakes in the paper, increased accessibility of the structure, and thinner electrodes (Fig. 4, A and B).

As shown in Fig. 4E, the use of Ti₃C₂T_x paper electrodes instead of multilayer exfoliated Ti₃C₂T_x in some electrolytes (e.g., KOH and NaOAc) roughly doubled the gravimetric capacitance (see fig. S8 for more information about the performance of Ti₃C₂T_x paper in NaOAc and MgSO₄). Even more impressively, the volumetric capacitance values recorded for few-layer Ti₃C₂T_x were on the order of 340 F/cm³ for KOH (Fig. 4C and fig. S9). Those values are much higher than those found for activated graphene [60 to 100 F/cm³ (15, 20)] or micrometer-thin carbide-derived carbon electrodes [180 F/cm³ (21, 22)]. Extreme values for MnO₂ hybrid electrodes [1200 F/cm³ (23); 640 F/cm³ (24)] were obtained on thin films of supported nanoparticles, and therefore cannot be compared with our electrodes. A capacitance retention test performed by galvanostatic cycling at 1 A/g showed almost no degradation in performance after 10,000 cycles (Fig. 4F).

Our results show that a variety of cations of various charges and sizes can readily intercalate, from aqueous solutions, both multilayer exfoliated Ti₃C₂T_x and MXene paper made of few layers of Ti₃C₂T_x. The phenomenon depends on pH and the nature of the cations. Extensive investigation of the electrochemical properties of the Ti₃C₂T_x in these aqueous electrolytes showed notable intercalation capacitances. The best performance was observed in basic solutions, such as KOH and NaOH for binder-free Ti₃C₂T_x paper. The latter is highly flexible and yielded volumetric capacitance of up to 350 F/cm³.

As noted, eight MXenes have been reported to date (10) and many more have been theoretically predicted (25, 26). Thus, the volumetric capacitances described above are probably far from the maximum values possible for MXenes

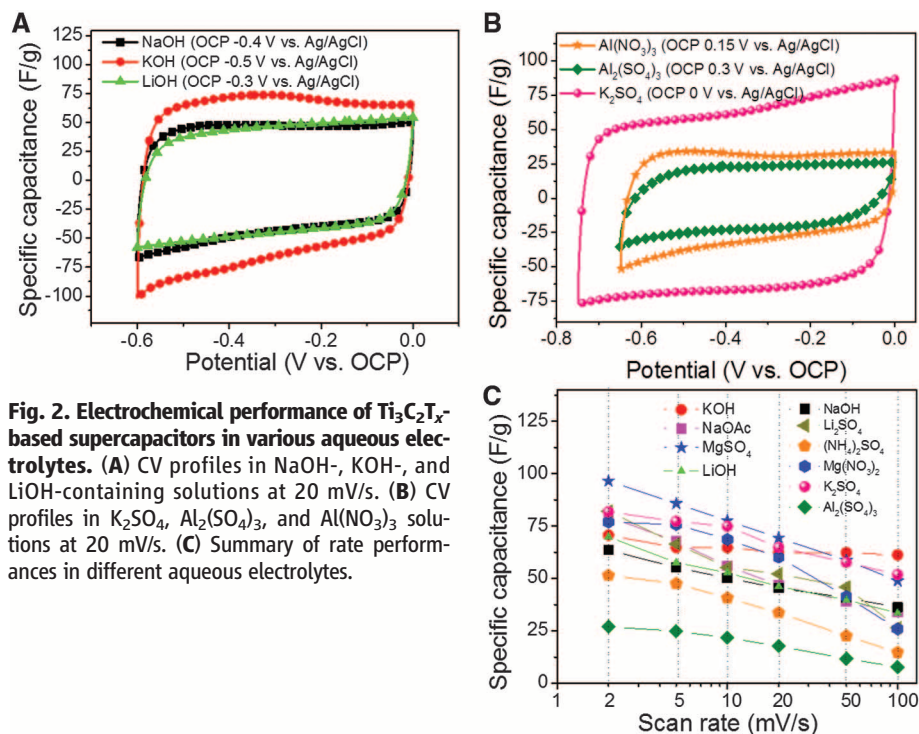


Fig. 2. Electrochemical performance of Ti₃C₂T_x-based supercapacitors in various aqueous electrolytes. (A) CV profiles in NaOH-, KOH-, and LiOH-containing solutions at 20 mV/s. (B) CV profiles in K₂SO₄, Al₂(SO₄)₃, and Al(NO₃)₃ solutions at 20 mV/s. (C) Summary of rate performances in different aqueous electrolytes.

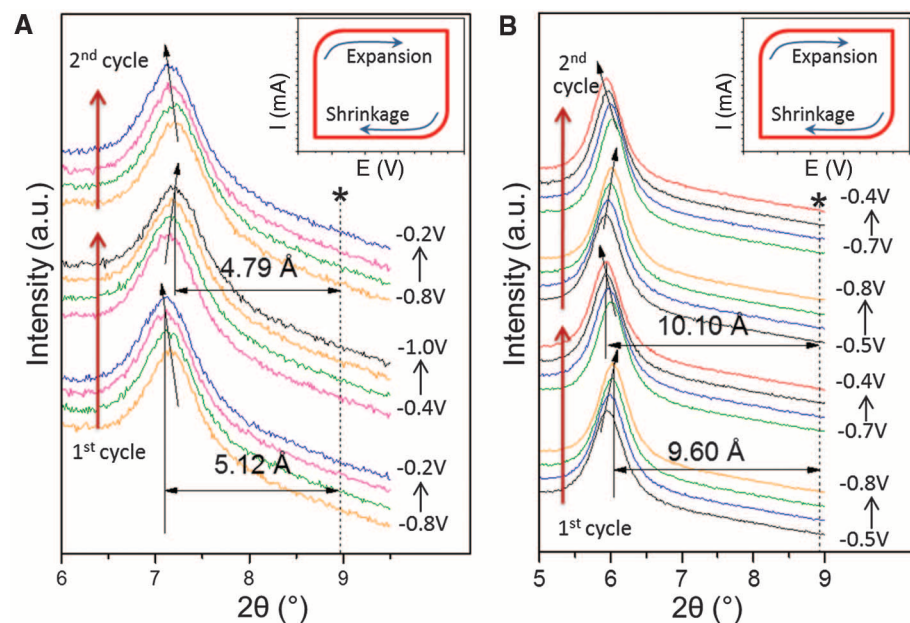
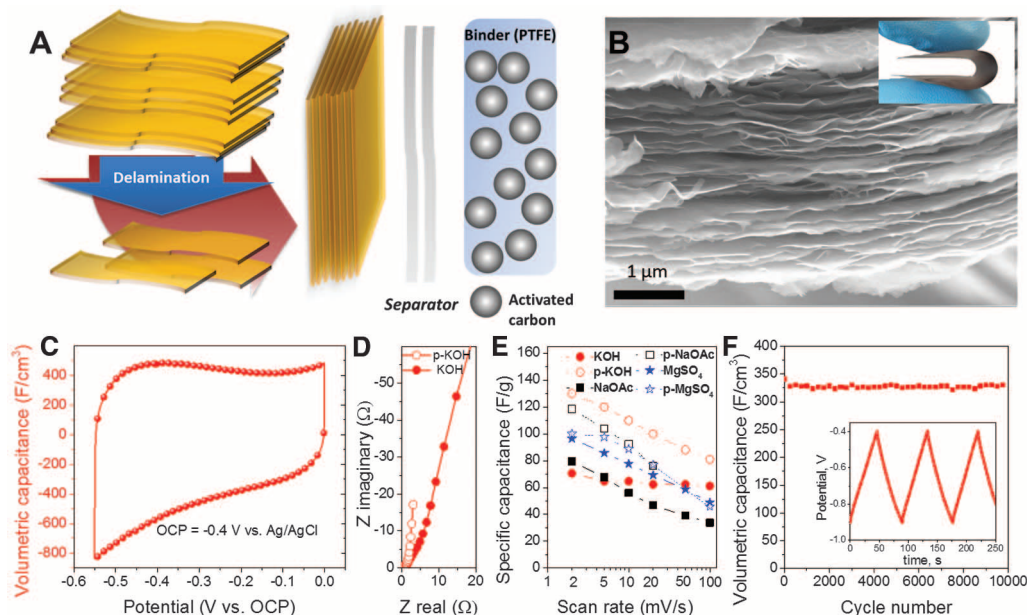


Fig. 3. Electrochemical in situ x-ray diffraction study of multilayer exfoliated Ti₃C₂T_x. (A) Ti₃C₂T_x in 1 M KOH solution; (B) Ti₃C₂T_x in 1 M MgSO₄ solution. Vertical dashed lines indicate the original position of the (0002) peak of the Ti₃C₂T_x electrodes before mounting in a cell. Inclined arrows show the direction of the (0002) peak shift. Insets illustrate cycling direction and concomitant changes in *c* lattice parameters during cycling. In both KOH and MgSO₄ electrolytes, shrinkage during cathodic polarization is observed.

in general. The flexibility of Ti₃C₂T_x paper (Fig. 4B) also opens the door for the use of MXenes in flexible and wearable energy storage devices (27). The fact that a variety of ions, as different as Na⁺ and Al³⁺, can be accommodated between the MXene layers may also enable MXene use

in batteries as well as in metal-ion capacitors (battery-supercapacitor hybrids). Thus, this work opens up exciting possibilities of developing improved intercalation electrodes for batteries, supercapacitors, and hybrid devices using a large variety of ions and/or electrode chemistries.

Fig. 4. Electrochemical performance of binder-free $\text{Ti}_3\text{C}_2\text{T}_x$ paper electrodes: (A) Schematic of electrode fabrication. During the first stage, the multilayer $\text{Ti}_3\text{C}_2\text{T}_x$ powders are delaminated to produce few-layer MXene flakes (see supplementary materials); the resulting colloidal solution is filtered through a porous membrane, producing binder- and additive-free $\text{Ti}_3\text{C}_2\text{T}_x$ paper electrodes for further use in capacitance tests. (B) Scanning electron micrograph of paper electrode. Inset is a photograph of the paper showing its flexibility. (C) CV of $\text{Ti}_3\text{C}_2\text{T}_x$ paper in KOH electrolyte. (D) EIS data in KOH for $\text{Ti}_3\text{C}_2\text{T}_x$ electrode (KOH, solid symbols) and $\text{Ti}_3\text{C}_2\text{T}_x$ paper (p-KOH). (E) Rate performance of the $\text{Ti}_3\text{C}_2\text{T}_x$ paper (open symbols) versus multilayer exfoliated $\text{Ti}_3\text{C}_2\text{T}_x$ electrode (solid symbols) in KOH-, MgSO_4 -, and NaOAc-containing electrolytes. (F) Capacitance retention test of $\text{Ti}_3\text{C}_2\text{T}_x$ paper in KOH. Inset: Galvanostatic cycling data collected at 1 A/g.



References and Notes

- B. Conway, *Electrochemical Supercapacitors: Scientific Fundamentals and Technological Applications* (Kluwer Academic/Plenum, New York, 1999).
- P. Simon, Y. Gogotsi, *Nat. Mater.* **7**, 845–854 (2008).
- I. E. Rauda, V. Augustyn, B. Dunn, S. H. Tolbert, *Acc. Chem. Res.* **46**, 1113–1124 (2013).
- K. Naoi, W. Naoi, S. Aoyagi, J. I. Miyamoto, T. Kamino, *Acc. Chem. Res.* **46**, 1075–1083 (2013).
- V. Augustyn et al., *Nat. Mater.* **12**, 518–522 (2013).
- Z. J. Sun et al., *J. Power Sources* **216**, 425–433 (2012).
- S. Stankovich et al., *Nature* **442**, 282–286 (2006).
- D. Li, M. B. Müller, S. Gilje, R. B. Kaner, G. G. Wallace, *Nat. Nanotechnol.* **3**, 101–105 (2008).
- M. W. Barsoum, T. El-Raghy, *Am. Sci.* **89**, 334 (2001).
- M. Naguib et al., *Adv. Mater.* **23**, 4248–4253 (2011).
- M. Naguib et al., *ACS Nano* **6**, 1322–1331 (2012).
- O. Mashtalir, M. Naguib, B. Dyatkin, Y. Gogotsi, M. W. Barsoum, *Mater. Chem. Phys.* **139**, 147–152 (2013).
- O. Mashtalir et al., *Nature Commun.* **4**, 1716 (2013).
- J. Come et al., *J. Electrochem. Soc.* **159**, A1368–A1373 (2012).
- Q. Tang, Z. Zhou, P. W. Shen, *J. Am. Chem. Soc.* **134**, 16909–16916 (2012).
- Y. Zhu et al., *Science* **332**, 1537–1541 (2011).
- L. Mai et al., *Sci. Rep.* **3**, 1718 (2013).
- P. W. Ruch et al., *Carbon* **48**, 1880–1888 (2010).
- O. Ghodbane, F. Ataherian, N.-L. Wu, F. Favier, *J. Power Sources* **206**, 454–462 (2012).
- S. Murali et al., *Nano Energy* **10**, 1016/j.nanoen.2013.01.007 (2013).
- J. Chmiola, C. Largeot, P.-L. Taberna, P. Simon, Y. Gogotsi, *Science* **328**, 480–483 (2010).
- M. Heon et al., *Energy Environ. Sci.* **4**, 135 (2011).
- X. Lang, A. Hirata, T. Fujita, M. Chen, *Nat. Nanotechnol.* **6**, 232–236 (2011).
- X. Zhao et al., *ACS Nano* **6**, 5404–5412 (2012).
- M. Kurtoglu, M. Naguib, Y. Gogotsi, M. W. Barsoum, *MRS Commun.* **2**, 133–137 (2012).
- M. Khazaei et al., *Adv. Funct. Mater.* **23**, 2185–2192 (2012).
- J. R. Miller, *Science* **335**, 1312–1313 (2012).

Acknowledgments: We thank B. Duployer and J. Come at Paul Sabatier University for help in electrochemical experiments, V. N. Mochalin and M. Beidaghi for helpful discussions, and the Centralized Research Facility of Drexel

University for access to XRD and SEM equipment. MXene synthesis was funded by the U.S. Department of Energy, Office of Vehicle Technologies, Batteries for Advanced Transportation Technologies (BATT) Program, under contract DE-AC02-05CH11231, subcontract 6951370. M.R.L. was supported by the U.S. Department of Energy, Energy Storage Systems Research Program, through Sandia National Laboratory. P.S. was supported by the European Research Council (advanced grant ERC-2011-AdG, project 291543-IONACES) and the Chair of Excellence “Embedded multi-functional nanomaterials” from the EADS Foundation. Collaboration between Drexel University and Paul Sabatier

University was supported by the Partner University Fund of the Embassy of France.

Supplementary Materials

www.sciencemag.org/content/341/6153/1502/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S9

Tables S1 to S4

References (28, 29)

5 June 2013; accepted 20 August 2013

10.1126/science.1241488

Shape Memory and Superelastic Ceramics at Small Scales

Alan Lai,¹ Zehui Du,² Chee Lip Gan,^{2,3} Christopher A. Schuh^{1*}

Shape memory materials are a class of smart materials able to convert heat into mechanical strain (or strain into heat) by virtue of a martensitic phase transformation. Some brittle materials such as intermetallics and ceramics exhibit a martensitic transformation but fail by cracking at low strains and after only a few applied strain cycles. Here we show that such failure can be suppressed in normally brittle martensitic ceramics by providing a fine-scale structure with few crystal grains. Such oligocrystalline structures reduce internal mismatch stresses during the martensitic transformation and lead to robust shape memory ceramics that are capable of many superelastic cycles up to large strains; here we describe samples cycled as many as 50 times and samples that can withstand strains over 7%. Shape memory ceramics with these properties represent a new class of actuators or smart materials with a set of properties that include high energy output, high energy damping, and high-temperature usage.

Shape memory materials are solid-state transducers, able to convert heat to strain and vice versa. They exhibit two unusual properties: (i) the shape memory effect, which is the ability to transform to a “remembered” predefined shape upon the application of heat, and (ii) superelasticity, which is the ability to deform to large strains recoverably, while dissipating energy as heat. The underlying mechanism in crystalline

shape memory materials is a thermoelastic martensitic transformation between two crystallographic phases that can be induced thermally (the shape memory effect) or by the application of stress (superelasticity) (1, 2). The ability to transduce heat and strain renders shape memory materials useful in a wide variety of actuation, energy-damping, and energy-harvesting applications (3–7). To be of practical use, the material must

be able to accommodate the extreme deviatoric strains associated with the phase transformation without cracking or otherwise sustaining damage. There are, however, a number of materials with martensitic transformations that do cause cracking, most often because shape distortions in adjacent crystal grains are incommensurate with one another, inducing large mismatch stresses and triggering fracture.

One conspicuous class of brittle materials—ceramics—falls into this category. A number of brittle ceramics exhibit martensitic transformations and thus are candidate shape memory materials (8–10). For example, zirconia has a well-studied martensitic transformation (8, 11–14) between tetragonal and monoclinic phases, with associated shear strains of up to 15% (15, 16). However, the mismatch stresses in polycrystalline zirconia prevent shape memory behavior. At strains of only about ~2%, cracking is observed after only a few transformation cycles (17). This is unlike shape memory metals such as Ni-Ti, which can withstand large strains up to ~8% and at lower strain levels can be reversibly transformed up to millions of cycles (18).

Because the fundamental cause of cracking in ceramics with martensitic transformations is transformation mismatch stresses, our strategy is to reduce those stresses through two main approaches. First, we reduce the size scale of the specimen itself: A smaller ceramic has a higher surface area-to-volume ratio, and the free surfaces can contribute to stress relaxation. Second, we strive to reduce the number of crystals within the volume of the specimen: An “oligocrystalline” or even single-crystalline material contains fewer grain junctions where the individual grain transformation strains will compete. Both of these concepts have been explored in shape memory metals (7, 19–21); we extend their application to ceramics.

Our experiments used polycrystalline zirconia doped with either ceria, yttria, or both, processed into solid polycrystalline specimens using conven-

tional methods (22, 23). From these polycrystalline specimens micrometer-scale pillars were fabricated, using focused ion-beam milling. The resulting pillars were sized to be smaller than or near the average grain size of the ceramics, and so were either single-crystalline or oligocrystalline [defined in (21) as having more surface area than grain boundary area] in every case. We applied loads to pillars both in a mode of axial compression and in bending, using a Hysitron nanomechanical test platform with a blunt conospherical tip (24).

A typical stress-strain curve for a 16 mole % (mol %) $\text{CeO}_2\text{-ZrO}_2$ superelastic specimen loaded in compression is shown in Fig. 1A. Loading begins in the tetragonal phase (which here, in the context of shape memory materials, is referred to as “austenite”), and after an initial linear elastic response, a critical stress is reached that induces the phase change into the monoclinic phase (which is referred to as “martensite”). This is seen as a slope reduction in the curve, which evolves as the transformation progresses to large strains of ~7%. Upon unloading, there is a linear elastic response of the martensite phase until another critical stress—that for reversion to austenite—is reached and a second lower plateau is seen. Complete unloading leads to a full recovery of the strain. The total energy dissipated during the cycle is quantified by the area within the curve and is listed in table S1.

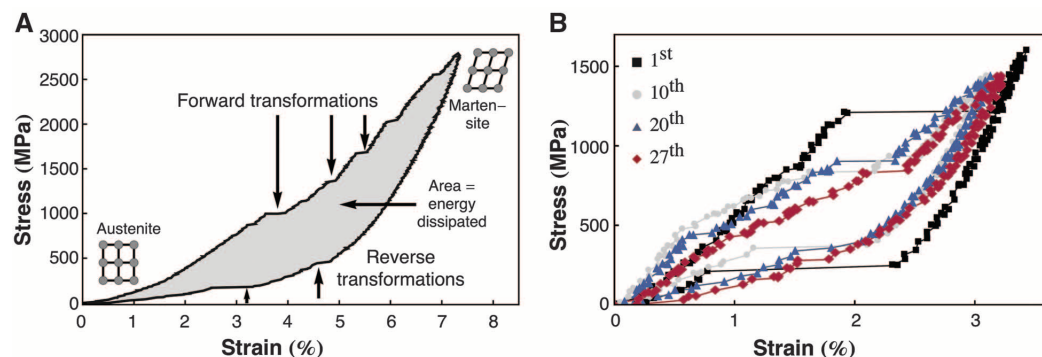
The stress-strain curve shown in Fig. 1A is characteristic of the many hundreds that we have measured on a variety of fine-scale austenitic zirconia ceramic pillars. We find that these samples exhibit all the attributes of a good superelastic material. This includes large strains up to 7% in some pillars, as well as the ability to cycle reversibly through the transformation many times. A series of typical stress-strain curves during cycling is shown in Fig. 1B and shows that superelastic properties are present over many cycles. There is a gradual evolution of the stress-strain hysteresis loop with cycling that is expected as a superelastic material becomes “trained” to a particular kinematic transformation pathway (25). In addition to the results in Fig. 1, a variety of other specimens were cyclically loaded without failing for dozens of cycles; up to 50 cycles were applied to a single pillar. More stress-strain curves

exhibiting a range of different achievable stress and strain combinations for different pillars can be found in figs. S4 to S11. Some pillars did experience cracking [see (24) for more details], but only after many more cycles than have previously been reported in polycrystalline specimens (17).

Fine-scale zirconia pillars in the tetragonal (austenite) phase were also found to exhibit excellent shape memory properties when deformed at a temperature in between the martensite and austenite transition temperatures, as shown in the scanning electron microscope images in Fig. 2. Upon loading at room temperature, shape change is effected by a stress-induced transformation from austenite to martensite, and when the load is removed the new shape remains. This is illustrated for an example pillar of 8 mol % $\text{CeO}_2\text{-0.5 mol % Y}_2\text{O}_3\text{-ZrO}_2$, loaded in a bending mode, between Fig. 2, A and B, to an approximate strain of 8% (24). The occurrence of the stress-induced martensitic transformation is also evidenced by stripe patterns formed on the lateral side of the pillar that we interpret as martensite variants, as shown in fig. S12A. Subsequent heating above the austenite transition temperature causes the martensite to revert to austenite, which is crystallographically constrained to return the material to its original shape. This shape recovery is shown in Fig. 2C after the specimen was heated to 500°C for 2 hours, and was accompanied by the nearly complete disappearance of the martensite variant stripe patterns on the side of the pillar (fig. S12B).

The results shown in Figs. 1 and 2 confirm that zirconia ceramics are capable of both the shape memory effect and superelasticity, and the demonstration of these effects to large strains and through multiple cycles departs substantially from the prior literature on the martensitic transformation in zirconia. As noted earlier, the cracking and failure of zirconia ceramics during such transformations are widely known. Cracking has only been suppressed in prior work by completely eliminating the transformation itself, as is done for the fully stabilized zirconia ceramics in wide technological usage today (8). Some earlier reports have suggested that shape memory properties may be possible in zirconia (17, 26–28), but these also reported failure at low strains (~1 to 2%) after only one or a few cycles.

Fig. 1. Demonstration of superelasticity, showing large fully recoverable strains and the ability to withstand cycling. (A) Micro-compression stress-strain curve for superelastic zirconia pillar [effective diameter $d_{\text{eff}} = 1.82 \mu\text{m}$, pillar B in (24)], in which the initial elastic loading of austenite is followed by forward transformation plateaus during the formation of martensite. This is followed by elastic unloading and reverse transformation plateaus with a reversion to austenite. **(B)** Stress-strain curves for a pillar with $d_{\text{eff}} = 1.19 \mu\text{m}$ [pillar G in (24)]. The superelastic response stabilizes after ~10 cycles.



¹Department of Materials Science and Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA.

²Temasek Laboratories, Nanyang Technological University (NTU), Singapore. ³School of Materials Science and Engineering, Nanyang Technological University, Singapore.

*Corresponding author. E-mail: schuh@mit.edu

The prospect of a new class of shape memory materials based on fine-scale ceramics is potentially technologically interesting, because ceramics occupy a different region in property space than any other shape memory materials.

The very high strength of ceramics permits access to shape memory and superelasticity at very high stress levels relative to shape memory

metals. Figure 3A shows a single superelastic curve for one of our zirconia ceramics as compared to literature data for two common shape memory alloys; the achievable actuation stress in the ceramic is four or more times that in the metals. A more thorough evaluation of the actuation stresses and strains of shape memory ceramics vis-à-vis other actuators of many kinds is

presented in Fig. 3B. Shape memory ceramics offer work output values approaching 100 MJ/m^3 and on this basis exceed not only shape memory alloys but also mechanical systems such as hydraulics.

Figure 3A also highlights another opportunity for shape memory ceramics in the area of energy damping. The amount of mechanical energy damped into heat is reflected by the hysteresis area in the stress-strain curves of Fig. 3A. Because the ceramic is stiffer and accesses larger stresses, it has a greater opportunity to expand the hysteresis area and can thus reversibly damp considerable mechanical energy as compared to shape memory metals. For reversible loading, we calculate a damping merit index for beams in bending or compression, merit index $= \eta \cdot E^{1/2}$, where E is the Young's modulus and η is the loss factor (24). For superelastic zirconia, we obtained merit index values of $\sim 2 \text{ GPa}^{1/2}$. This value is about double that reported in Cu-Al-Ni microscale shape memory alloys (7).

Another property axis on which ceramics are generally differentiated from metals is temperature; ceramics are generally more refractory than metals, and as such, shape memory ceramics should be thought of as a class of potential high-temperature shape memory materials. Through tuning of the composition, zirconia ceramics can exhibit transformation temperatures ranging from 0° to 1200°C (fig. S3). This is in contrast to shape memory metals, which have maximum transformation temperatures of $\sim 500^\circ\text{C}$; a graphical comparison is made in Fig. 3C.

A final interesting prospect for the use of shape memory ceramics is as a structural material subject to a one-time mechanical loading event, in which repeatable transformations are not required. In such cases, the ceramic can be loaded all the way to failure, and unlike a conventional brittle ceramic, the superelastic ceramic can exhibit substantial apparent ductility (or malleability) before fracture, by virtue of the phase transformation. Figure 3D illustrates this with two compressive stress-strain curves for zirconia. The classical response achieves elastic strains of 1 to 2% with some limited incipient plasticity just before brittle fracture, but the shape memory ceramic transforms, dissipating energy through a strain of about 7% in this single example, with theoretical uniaxial transformation strains up to 10% possible, depending on crystallographic orientation. The result is a ceramic that dissipates exceptionally large amounts of energy upon fracture, $\sim 35 \text{ MJ/m}^3$, approaching the magnitude of the energy dissipated by structural metals. The combination of ceramic strength levels with apparent ductility offers interesting opportunities in mechanical design.

Although much work remains to optimize these materials for any proposed application, the unusual properties of the shape memory ceramics reported here, as well as other ceramics that exhibit martensitic transformations (10) and may thus exhibit similar properties, offer a strong motivation to pursue their development.

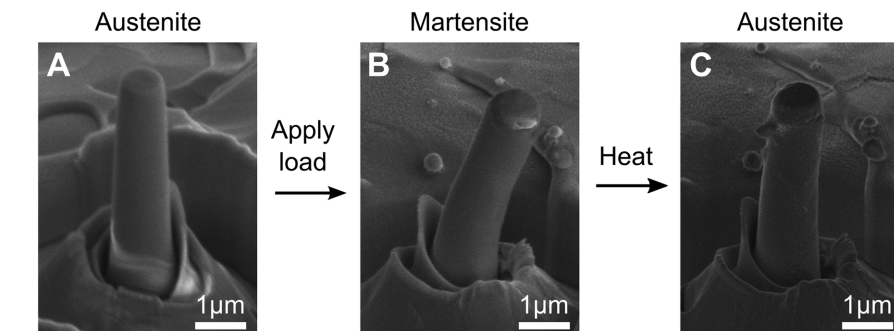


Fig. 2. Demonstration of the shape memory effect in small-scale zirconia pillars. (A) Pillar milled from austenite before deformation [$d_{\text{eff}} = 1.02 \mu\text{m}$, pillar] in (24)] (B) Pillar after bending at room temperature in the martensite phase. (C) Heating induces the phase transformation to austenite and leads to recovery of the original shape, which is retained upon cooling to a state comparable to that in (A).

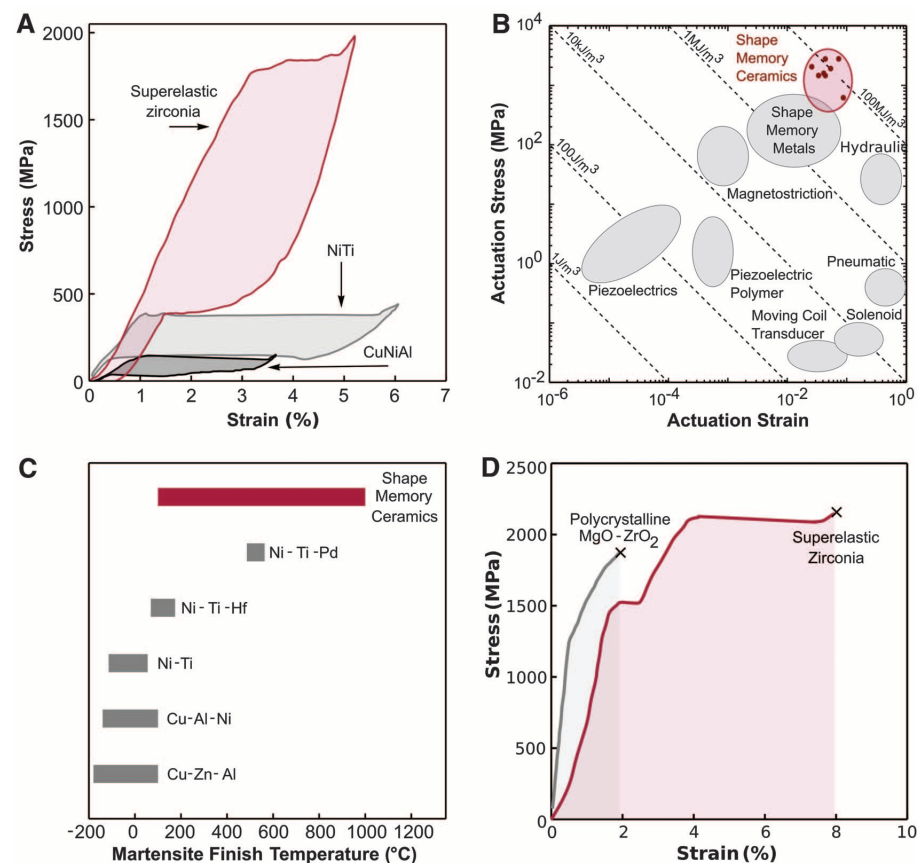


Fig. 3. Comparison of small-scale shape memory zirconia with other structural and actuator materials. (A) Stress-strain curves for superelastic zirconia, NiTi (29), and CuAlNi (7). (B) Actuator stress and strain for various actuator materials and systems. Contours of constant specific work are indicated by dashed lines [adapted from (3)]. (C) Martensite transformation temperatures for shape memory ceramics and metals (30); ceramics have much higher operational ranges. (D) Stress-strain curves for superelastic zirconia and MgO partially stabilized zirconia (31); upon loading to fracture, significantly more energy is dissipated in the fine-scale superelastic zirconia.

References and Notes

- T. Tadaki, K. Otsuka, K. Shimizu, *Annu. Rev. Mater. Sci.* **18**, 25–45 (1988).
- K. Otsuka, C. M. Wayman, *Shape Memory Materials* (Cambridge Univ. Press, Cambridge, 1999).
- J. E. Huber, N. A. Fleck, M. F. Ashby, *Proc. R. Soc. London Ser. A* **453**, 2185–2205 (1997).
- M. Zupan, M. F. Ashby, N. A. Fleck, *Adv. Eng. Mater.* **4**, 933–939 (2002).
- J. Ma, I. Karaman, *Science* **327**, 1468–1469 (2010).
- A. L. Robinson, *Science* **191**, 934–936 (1976).
- J. San Juan, M. L. Nó, C. A. Schuh, *Nat. Nanotechnol.* **4**, 415–419 (2009).
- P. M. Kelly, L. R. Francis Rose, *Prog. Mater. Sci.* **47**, 463–557 (2002).
- S. H. Kirby, L. A. Stern, *J. Struct. Geol.* **15**, 1223–1240 (1993).
- W. M. Kriven, *J. Am. Ceram. Soc.* **71**, 1021–1030 (1988).
- H. Cai, K. T. Faber, *Scr. Metall. Mater.* **28**, 1161–1166 (1993).
- M. E. Launey, R. O. Ritchie, *Adv. Mater.* **21**, 2103–2110 (2009).
- M. Rühle, A. G. Evans, *Prog. Mater. Sci.* **33**, 85–167 (1989).
- A. H. Heuer, M. Rühle, D. B. Marshall, *J. Am. Ceram. Soc.* **73**, 1084–1093 (1990).
- N. K. Simha, *J. Mech. Phys. Solids* **45**, 261–292 (1997).
- M. V. Swain, *Nature* **322**, 234–236 (1986).
- P. E. Reyes-Morel, J. S. Cherng, I. W. Chen, *J. Am. Ceram. Soc.* **71**, 648–657 (1988).
- A. R. Pelton, *J. Mater. Eng. Perform.* **20**, 613–617 (2011).
- Y. Chen, C. A. Schuh, *Acta Mater.* **59**, 537–553 (2011).
- N. Ozdemir, I. Karaman, N. A. Mara, Y. I. Chumlyakov, H. E. Karaca, *Acta Mater.* **60**, 5670–5685 (2012).
- S. M. Ueland, Y. Chen, C. A. Schuh, *Adv. Funct. Mater.* **22**, 2094–2099 (2012).
- J. G. Duh, H. T. Dai, W. Y. Hsu, *J. Mater. Sci.* **23**, 2786–2791 (1988).
- T. S. Sheu, T. Y. Tien, I. W. Chen, *J. Am. Ceram. Soc.* **75**, 1108–1116 (1992).
- Materials and methods are available as supplementary materials on Science Online.
- J. San Juan, M. L. Nó, C. A. Schuh, *Acta Mater.* **60**, 4093–4106 (2012).
- B. Jiang et al., in *Materials Research Society Symposium Proceedings* (Materials Research Society, Warrendale, PA, 1992), vol. 246, pp. 213–216.
- X. J. Jin, *Curr. Opin. Solid State Mater. Sci.* **9**, 313–318 (2005).
- Y. Zhang, X. Jin, X. Z. Hsu, Y. Zhang, J. Shi, *Mater. Sci. Forum* **394–395**, 573–576 (2002).
- A. Runciman, D. Xu, A. R. Pelton, R. O. Ritchie, *Biomaterials* **32**, 4987–4993 (2011).
- D. Lagoudas, *Shape Memory Alloys Modeling and Engineering Applications* (Springer, New York, 2008).
- J. Lankford, R. A. Page, L. Rabenberg, *J. Mater. Sci.* **23**, 4144–4156 (1988).

Acknowledgments: We thank A. Schwartzmann, S. Speakman, and S. Chen at MIT's Center for Materials Science and Engineering; D. Galler at MIT; and S. Amini and A. Miserez at NTU for their assistance with experiments, as well as N. Antoniou at Harvard Center for Nanoscale Systems. We acknowledge the project funding support under project agreements 9011102294 and 9011102296. MIT has applied for a patent, application number USSN 13/791,857, dated 2 July 2012; and MIT/NTU jointly applied for a provisional patent, application number USSN 61/775,446, dated 8 March 2013, related to the materials and design methods produced in this work. We acknowledge the late Prof. Ma Jan (NTU) for initiating this project collaboration.

Supplementary Materials

www.sciencemag.org/content/341/6153/1505/suppl/DC1

Materials and Methods

Figs. S1 to S12

Table S1

References (32–34)

29 April 2013; accepted 20 August 2013

10.1126/science.1239745

Near-Complete Extinction of Native Small Mammal Fauna 25 Years After Forest Fragmentation

Luke Gibson,^{1*} Antony J. Lynam,² Corey J. A. Bradshaw,³ Fangliang He,^{4,5*} David P. Bickford,^{1*} David S. Woodruff,⁶ Sara Bumrungsri,⁷ William F. Laurance⁸

Tropical forests continue to be felled and fragmented around the world. A key question is how rapidly species disappear from forest fragments and how quickly humans must restore forest connectivity to minimize extinctions. We surveyed small mammals on forest islands in Chiew Larn Reservoir in Thailand 5 to 7 and 25 to 26 years after isolation and observed the near-total loss of native small mammals within 5 years from <10-hectare (ha) fragments and within 25 years from 10- to 56-ha fragments. Based on our results, we developed an island biogeographic model and estimated mean extinction half-life (50% of resident species disappearing) to be 13.9 years. These catastrophic extinctions were probably partly driven by an invasive rat species; such biotic invasions are becoming increasingly common in human-modified landscapes. Our results are thus particularly relevant to other fragmented forest landscapes and suggest that small fragments are potentially even more vulnerable to biodiversity loss than previously thought.

Rapid deforestation poses a major threat to one of the planet's greatest bastions of biodiversity, tropical forests (1–4). Whether by chance or design, small fragments of forest typically persist in the aftermath of deforestation, effectively islands within a sea of agriculture, urbanization, or other modified lands that are unsuitable for most forest species (5, 6). Many of the species that originally occupied the forest will disappear from these isolated fragments, but this loss occurs over a relaxation period until a new, more depauperate equilibrium community is reached (7). The number of species that will ultimately disappear from a forest fragment—its “extinction debt” (8)—will vary based on the size of the fragment, its surrounding habitat, the vagility of its constituent and neighboring species, and its distance from source populations (9).

Extinctions can be averted by reducing deforestation rates and reforesting fragmented forest landscapes (10). However, it remains uncertain how quickly such actions must be taken and what minimum fragment size is required to maintain functioning biotic communities. For 1000-ha forest fragments in Kenya, half of the total bird extinctions are projected to occur within 50 years, giving conservationists some time to mitigate conditions in large fragments (11). However, for smaller fragments, relaxation times are generally much more rapid (12, 13), and for ≤100-ha fragments, half of their original species can disappear within 15 years (14). Most studies of extinctions from forest fragments have focused on birds (11–14), and little is known about the sensitivity of other taxonomic groups.

We surveyed small mammals on forest islands in a reservoir five separate times after isolation to

assess the rate of species loss from forest fragments. Reservoirs can form useful natural laboratories to estimate extinction rates from isolated forest patches (15, 16). Chiew Larn Reservoir in southern Thailand was formed in 1986–1987 when 165 km² of forest was flooded, creating over 100 islands in the process (Fig. 1) (17). We selected 16 islands in the reservoir ranging from 0.3 to 56.3 ha in area and surveyed small mammal communities 5 to 7 years (for 12 of the 16 islands) and 25 to 26 years after isolation. Chiew Larn Reservoir is surrounded by two protected areas that form part of the largest (>3500 km²) contiguous forest area in southern Thailand. All surveyed islands were unoccupied by humans (18).

We found that native small mammal communities disappeared rapidly after fragmentation. By 5 to 7 years after fragmentation, three large islands in our sample (10 to 56 ha) sustained 7 to 12 species of small mammals (Table 1), which was similar to diversity found on the nearby mainland (table S3) (19). However, on nine small islands (<10 ha), species richness rapidly declined to

¹Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore.

²Wildlife Conservation Society, Global Conservation Programs, 2300 Southern Boulevard, Bronx, NY 10460, USA. ³The Environment Institute and School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia. ⁴YSU-Alberta Joint Lab for Biodiversity Conservation, State Key Laboratory of Biocontrol and School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275, China.

⁵Department of Renewable Resources, University of Alberta, Edmonton, Alberta T6G 2H1, Canada. ⁶Ecology, Behavior and Evolution Section, Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA. ⁷Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. ⁸Centre for Tropical Environmental and Sustainable Sciences and School of Marine and Tropical Biology, James Cook University, Cairns, Queensland 4878, Australia.

*Corresponding author. E-mail: lgibson@nus.edu.sg (L.G.); the@mail.sysu.edu.cn (F.H.); dbsbdp@nus.edu.sg (D.P.B.)

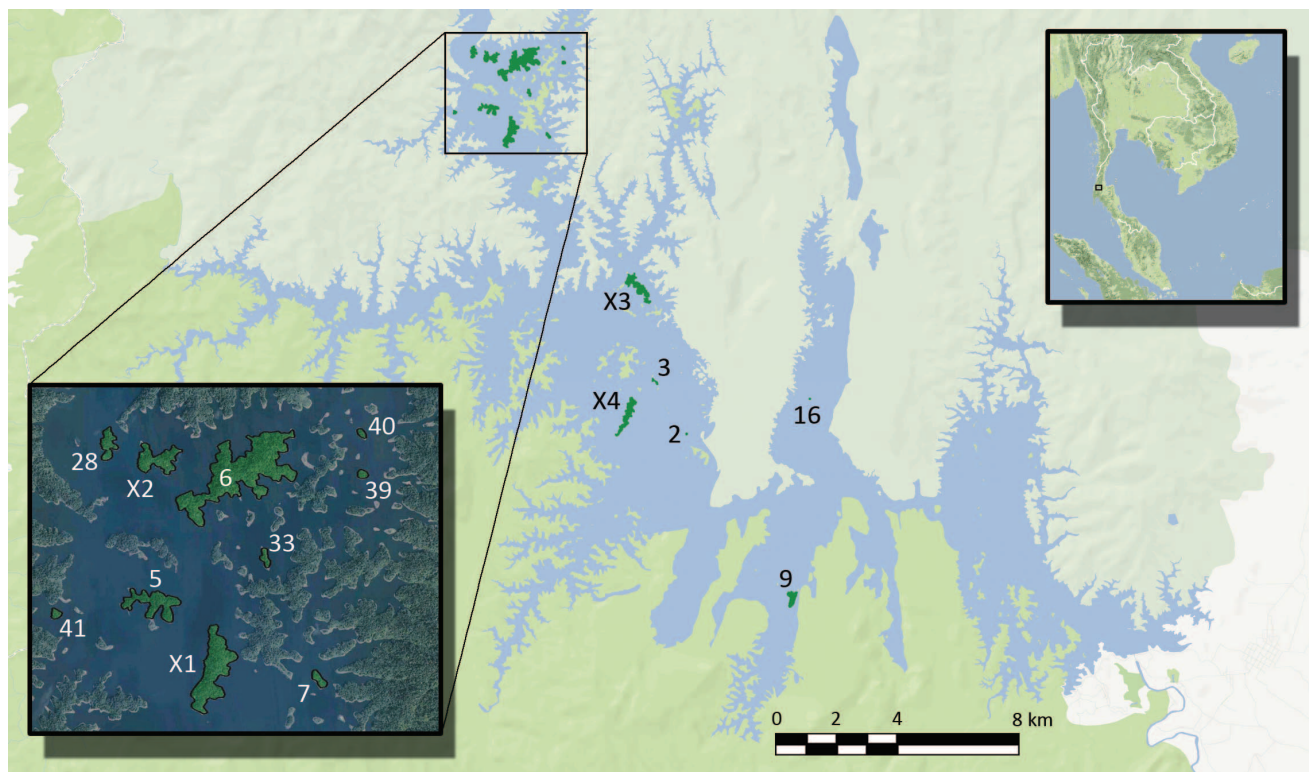


Fig. 1. Islands sampled in Chiew Larn Reservoir, Thailand. The reservoir is surrounded by protected forest areas in Khao Sok National Park to the south and west (shaded dark green) and Khlong Saeng Wildlife Sanctuary to the north and east (shaded light green). The 12 islands sampled during all surveys

are labeled by island number [in the sense of (19)], and the additional four islands surveyed in more recent surveys are labeled X1 to X4. The dam is located in the lower right corner of the figure. The location of the reservoir in southern Thailand is shown in the regional map inset.

Table 1. Number of small mammal species found on islands 5 to 7 years and 25 to 26 years after isolation. The maximum number of species observed on each island is reported. Islands X1 to X4 were only surveyed 25 to 26 years after isolation.

Island	Area (ha)	Richness (5 to 7 years)	Richness (25 to 26 years)
6	56.3	12	5
5	12.1	9	3
9	10.4	7	1
28	4.7	2	2
7	1.9	3	2
33	1.7	1	1
3	1.4	2	1
41	1.1	3	1
39	1.0	3	1
40	0.8	2	1
2	0.4	2	1
16	0.3	2	1
X1	23.5		2
X2	10.1		2
X3	24.4		2
X4	21.2		1

just one to three species during these initial surveys. After 25 to 26 years, native small mammals had virtually disappeared from all 16 islands (Fig. 2, figs. S1 and S2, Table 1, and table S1).

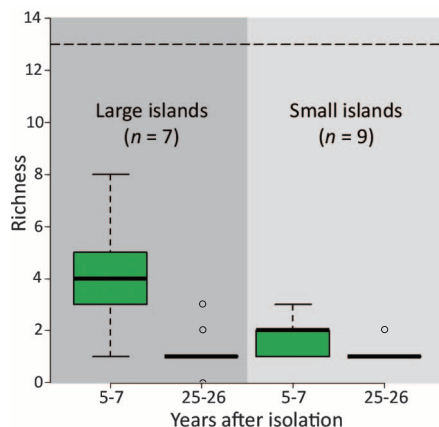


Fig. 2. Small mammal species richness per transect in large (10.1 to 56.3 ha, $n = 7$) and small (0.3 to 4.7 ha, $n = 9$) islands 5 to 7 years and 25 to 26 years after isolation. Plotted are median values, interquartile ranges, and full ranges (outliers are plotted as open circles). The upper dashed line represents the number of small mammal species found in surrounding mainland forest (table S3).

Species richness on islands was most strongly correlated with area, but there was an important contribution of time since isolation, as well as a weak negative interaction between area and time since isolation (table S2). We also surveyed small

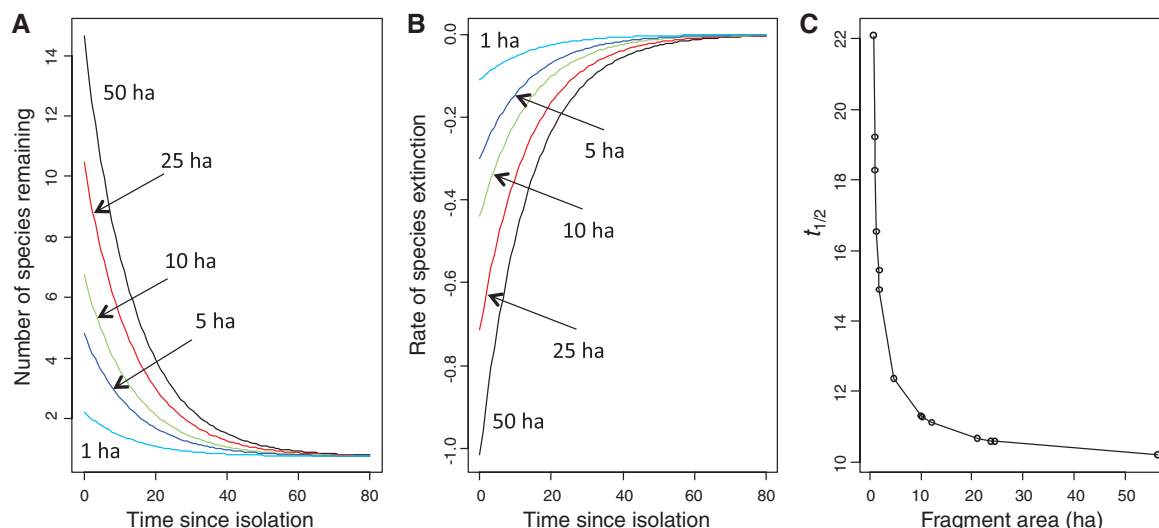
mammal communities in the mainland forest surrounding the reservoir and detected no similar decrease in diversity (table S3).

To describe the process of biotic relaxation over time for our islands of different size, we derived an island biogeographic model. The model included both immigration and extinction, which are negatively and positively proportional to the number of species on the islands, respectively (18). The model estimates the number of species occupying forest fragments before isolation and calculates the rate of extinction. The best-fitting model was

$$S_t = 0.751 - (0.751 - 2.223a^{0.482})e^{-0.0732t} \quad (1)$$

where S_t is the number of species at time t , a is island area, and t is time since isolation. This model fitted our data well (coefficient of determination = 0.783, fig. S3) and predicted that, before isolation, the largest island (56 ha) had 15.5 species and the smallest island (0.3 ha) had 1.2 species. For all islands, the mean time to extinction of half of the resident species ($t_{1/2}$) was 13.9 ± 3.9 years. Full relaxation to just one species was projected to occur within 40 years, regardless of fragment size (Fig. 3A). To determine the sensitivity of these results to the choice of underlying model, we also constructed a variant of the extinction-immigration component and considered two other species-area relationship models (Kobayashi

Fig. 3. Loss of species from forest fragments of various sizes based on model (1). (A and B) Number of species remaining and number of species lost per year on fragments after time (in years) since isolation. **(C)** Time to extinction of half of the species ($t_{1/2}$) initially present on forest fragments according to fragment area.



and Gleason) in addition to the Arrhenius power-law model; results differed little from those derived above (18). Our modeled extinction rates are similar to those observed by Ferraz *et al.* (14), who found that 100-ha forest fragments in the central Amazon lost half of their understory bird species in less than 15 years. However, our results diverge with the unexpected finding that species diversity declined faster on larger than on smaller islands (Fig. 3B), with the former having a shorter extinction half-life (Fig. 3C). This is a consequence of the catastrophic faunal collapse we documented across the entire archipelago of fragments, so that larger islands, which initially sustained the most species and the most forest specialists, exhibited the most rapid rate of species loss.

With few exceptions, only the Malayan field rat *Rattus tiomanicus* remained on islands 25 years after isolation (table S1). Commensal rodents such as *R. tiomanicus* are among the most common invasive species worldwide and often have a devastating effect on native fauna, particularly birds (20–23). *R. tiomanicus* does not occur naturally in undisturbed tropical forests of Southeast Asia, but is common in secondary forests, agricultural areas, and villages (24) and probably spread from such habitats to islands and reservoir fringes after inundation. By the time we had resurveyed islands 25 to 26 years after isolation, few native small mammals remained (just 13 and 9 individuals, respectively, were detected on the 16 sampled islands, not including the invading *R. tiomanicus*; table S1). At that point, all islands were dominated by the invasive rodent and if not already in ecological meltdown (25), were well on their way to becoming *Rattus* monocultures.

Small forest fragments can play important conservation roles in some contexts (26), including enhancing landscape connectivity and sustaining locally endemic species in regions such as Madagascar and the Brazilian Atlantic Forest, where most native vegetation has vanished. In these highly fragmented regions, however, mam-

malian communities can disappear rapidly, as has been observed for medium- and large-sized mammals in the Brazilian Atlantic Forest (27). In fact, regional estimates of extinctions from deforestation are probably worse than previously thought, because studies applying species-area curves have assumed that the persisting forest was contiguous (28). Additionally, exotic species such as *R. tiomanicus* are rapidly expanding into human-transformed and regenerating forest landscapes that increasingly dominate many tropical regions (29) and appear capable of sharply accelerating extinction rates in some fragmented landscapes. Hence, our findings, in which fragments were invaded and evidently profoundly destabilized by an invasive species, have considerable relevance for nature conservation in fragmented habitats globally. The apparent synergism between habitat fragmentation and species invasion underscores a dire need to maintain large intact forest blocks to sustain tropical biodiversity (4, 30).

References and Notes

1. F. Achard *et al.*, *Science* **297**, 999–1002 (2002).
2. R. Dirzo, P. H. Raven, *Annu. Rev. Environ. Resour.* **28**, 137–167 (2003).
3. G. P. Asner, T. K. Rudel, T. M. Aide, R. Defries, R. Emerson, *Conserv. Biol.* **23**, 1386–1395 (2009).
4. L. Gibson *et al.*, *Nature* **478**, 378–381 (2011).
5. E. N. Broadbent *et al.*, *Biol. Conserv.* **141**, 1745–1757 (2008).
6. W. F. Laurance, M. Goosman, S. G. Laurance, *Trends Ecol. Evol.* **24**, 659–669 (2009).
7. J. M. Diamond, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3199–3203 (1972).
8. D. Tilman, R. M. May, C. L. Lehman, M. A. Nowak, *Nature* **371**, 65–66 (1994).
9. L. R. Prugh, K. E. Hodges, A. R. E. Sinclair, J. S. Brashares, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 20770–20775 (2008).
10. O. R. Wearn, D. C. Reuman, R. M. Ewers, *Science* **337**, 228–232 (2012).
11. T. M. Brooks, S. L. Pimm, J. O. Oyugi, *Conserv. Biol.* **13**, 1140–1150 (1999).
12. J. M. Halley, Y. Iwasa, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 2316–2321 (2011).
13. W. F. Laurance *et al.*, *Biol. Conserv.* **144**, 56–67 (2011).
14. G. Ferraz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14069–14073 (2003).
15. J. Diamond, *Science* **294**, 1847–1848 (2001).
16. K. J. Feeley, J. W. Terborgh, *Anim. Conserv.* **11**, 366–368 (2008).
17. S. Nakhasathien, *Oryx* **23**, 146–154 (1989).
18. Materials and methods are available as supplementary materials on Science Online.
19. A. J. Lynam, I. Billick, *Biol. Conserv.* **91**, 191–200 (1999).
20. T. M. Blackburn, P. Cassey, R. P. Duncan, K. L. Evans, K. J. Gaston, *Science* **305**, 1955–1958 (2004).
21. D. B. Harris, D. W. Macdonald, *Ecology* **88**, 2330–2344 (2007).
22. H. P. Jones *et al.*, *Conserv. Biol.* **22**, 16–26 (2008).
23. D. B. Harris, *Biol. Invasions* **11**, 1611–1630 (2009).
24. C. M. Francis, *A Field Guide to the Mammals of Thailand and South-East Asia* (Asia Books, Bangkok, Thailand, 2008).
25. J. Terborgh *et al.*, *Science* **294**, 1923–1926 (2001).
26. I. M. Turner, R. T. Corlett, *Trends Ecol. Evol.* **11**, 330–333 (1996).
27. G. R. Canale, C. A. Peres, C. E. Guidorizzi, C. A. Gatto, M. C. M. Kierulff, *PLoS ONE* **7**, e41671 (2012).
28. I. Hanski, G. A. Zurita, M. I. Bellocq, J. Rybicki, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 12715–12720 (2013).
29. E. C. Ellis, K. K. Goldewijk, S. Siebert, D. Lightman, N. Ramankutty, *Glob. Ecol. Biogeogr.* **19**, 589–606 (2010).
30. W. F. Laurance *et al.*, *Nature* **489**, 290–294 (2012).

Acknowledgments: We thank our Thai field assistants for their invaluable efforts; the National Research Council of Thailand and the Department of National Parks, Wildlife and Plant Conservation for granting access and collection permission; and the superintendents and staff of Khlong Saeng Wildlife Sanctuary and Khao Sok National Park for access to Chiew Larn reservoir. We thank J.-F. Cosson for genetically confirming the identity of *Rattus tiomanicus*. Finally, we are indebted to N. Sodhi for providing this research opportunity on extinction debt. Field research in 1992–1994 was funded by grants BSR-9000486 and BSR-9300182 from NSF to D.S.W. Field research in 2012–2013 was funded by grant R-154-000-479-112 from the National University of Singapore and grant R-154-000-521-720 from the Ah Meng Memorial Conservation Fund. L.G. was supported by a Singapore International Graduate Award, C.J.A.B. by an Australian Research Council (ARC) Future Fellowship, F.H. by the National Sciences and Engineering Research Council of Canada and Sun Yat-sen University, and W.F.L. by an ARC Australian Laureate Fellowship. This study was approved by the Institutional Animal Care and Use Committee of the National University of Singapore.

Supplementary Materials

www.sciencemag.org/content/341/6153/1508/suppl/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 to S3
References (31–38)

14 May 2013; accepted 29 August 2013
10.1126/science.1240495

Safeguards for Cell Cooperation in Mouse Embryogenesis Shown by Genome-Wide Cheater Screen

Marion Dejosez,^{1,2,3} Hiroki Ura,³ Vicky L. Brandt,¹ Thomas P. Zwaka^{1,2,3*}

Ensuring cooperation among formerly autonomous cells has been a central challenge in the evolution of multicellular organisms. One solution is monoclonality, but this option still leaves room for exploitative behavior, as it does not eliminate genetic and epigenetic variability. We therefore hypothesized that embryonic development must be protected by robust regulatory mechanisms that prevent aberrant clones from superseding wild-type cells. Using a genome-wide screen in murine induced pluripotent stem cells, we identified a network of genes (centered on *p53*, *topoisomerase 1*, and olfactory receptors) whose down-regulation caused the cells to replace wild-type cells in vitro and in the mouse embryo—without perturbing normal development. These genes thus appear to fulfill an unexpected role in fostering cell cooperation.

Multicellularity has evolved independently a number of times. Under certain conditions, it offers selective advantages that outweigh the costs of constraining competition (1). It can afford protection against predation, increase metabolic efficiency through division of labor, promote more efficient resource consumption, and even create a defense against

noncooperative individuals (1–3). Cooperation, then, can be both a means and a motivation for the rise of multicellular organisms.

Once multicellularity is achieved, however, a conflict can emerge between organismal and cellular fitness (4–6). For example, among the facultative multicellular organisms myxobacteria and *Dictyostelium discoideum*, cells with “de-

factor” or “cheater” mutations (which produce more than their fair share of spores) can destroy the entire community of cells unless it is able to re-equilibrate toward cooperation (7–9). In the obligatory multicellular organism *Drosophila melanogaster*, cells with two different genotypes within a developmental niche can vie for occupancy; in most cases, the wild-type cells remove mutant cells that would reduce fitness at the level of the whole organism (10–13). Although it is reasonable to assume that normal mammalian development relies on coordinated responses within the organism to curtail the autonomous behavior of mutant cells, this prediction has not been tested by systematic screening for cheater or competitor mutations.

Cheater mutants have been identified in *D. discoideum* by genetic screening in which a mutagenized cell population was forced to pass through the three canonical stages of the amoeba life cycle: growth, development, and germination (7). We transposed this approach to an in vitro

¹Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ²Department of Developmental and Regenerative Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ³Baylor College of Medicine, Houston, TX 77030, USA.

*Corresponding author. E-mail: thomas.zwaka@mssm.edu

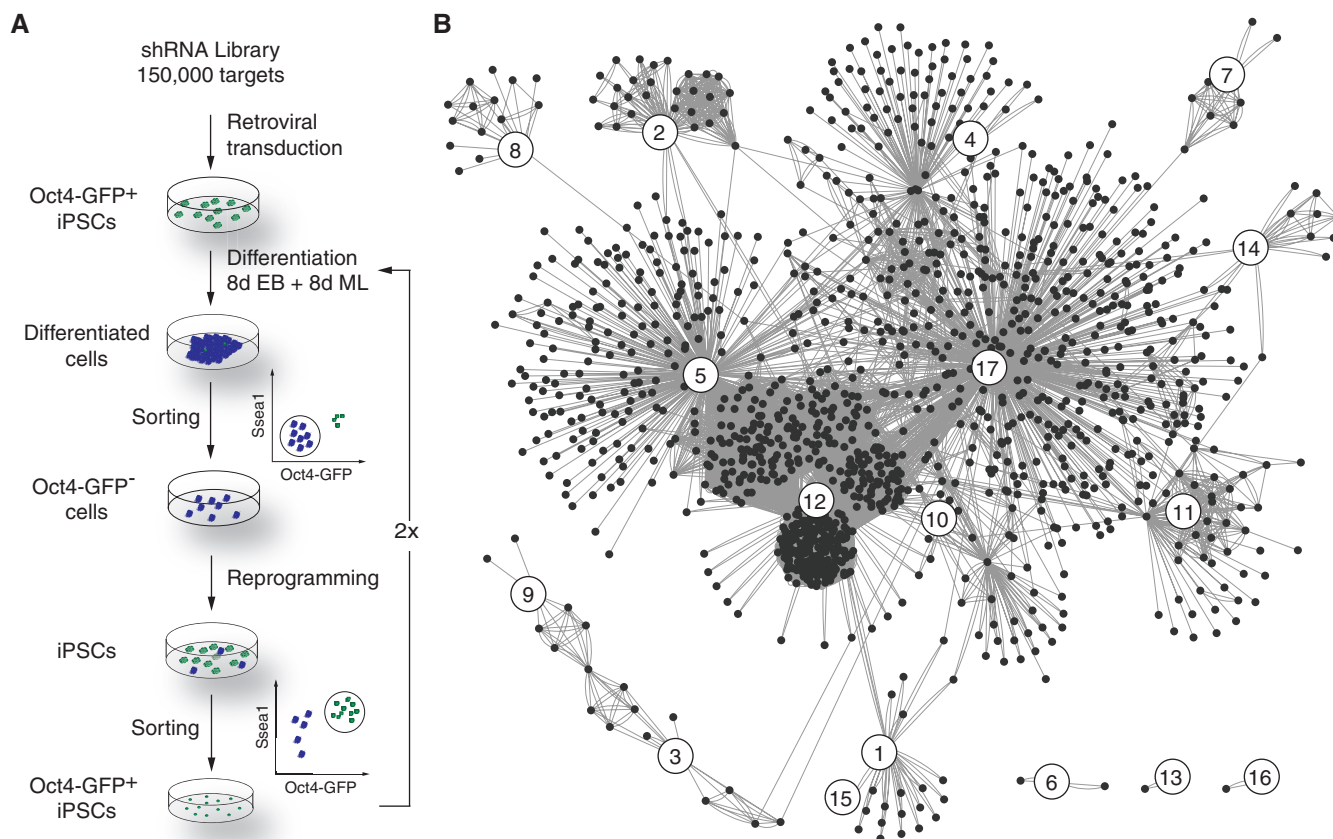


Fig. 1. Cell cheater screen. (A) Schematic of the screen used to identify candidate genes involved in cell cooperation, i.e., those whose knockdown confers a competitive advantage on stem cells. ML, monolayer. SSEA-1 and Oct4 are markers of pluripotency and self-renewal. (B) A cytoscape analysis of all candidate

genes identified at least twice and for which network information was available shows that many of the candidate genes are tightly linked to each other. 1, *Mpp3*; 2, *Ptfr*; 3, *Galk1*; 4, *Dbc1*; 5, *Csnk2a1*; 6, *Ltb4r1*; 7, *Bet1*; 8, *Cat*; 9, *Gcat*; 10, *Hspa5*; 11, *Casp8*; 12, *Top1*; 13, *Lmtk3*; 14, *Bmf*; 15, *Crabp1*; 16, *Dnajc2*; 17, *p53*.

model of murine embryogenesis by introducing a library of small inhibitory (hairpin) RNAs into induced pluripotent stem cells [iPSCs, cell line PF159 (14)] and reasoning, by analogy to the *D. discoideum* screen, that egoistical phenotypes would be more likely to emerge among mutantized cells under the considerable selective pressure of repeated rounds of growth [embryonic stem cell (ESC) expansion], development [embryoid body (EB) differentiation], and reacquisition of pluripotency (iPSCs) (15–19) (Fig. 1A; figs. S1 to S3; and see Methods for more detail).

Using this screen to select for cells that supersede wild-type cells simultaneously in all three categories (growth, differentiation, and repro-

gramming) in mixed populations of cells in a somatic context, we identified 131 putative cheater clones, a subset of which showed marked enrichment (fig. S4 and table S1). Gene ontology (GO) analyses revealed that a significant proportion of the candidate genes represent a relatively narrow set of functional categories, including cell communication, calcium channel activity, and kinase activity (fig. S4C and table S2). Inspection of table S1 reveals a number of notable genes, such as *p53*, *Top1* (topoisomerase 1), *Ptafr* (platelet-activating factor receptor), *Olfr1387* (olfactory receptor 1387), *Olfr305* (olfactory receptor 205), *Dcb1* (deleted in bladder cancer 1), *Casp8* (caspase 8), *Mpp3* (membrane protein,

palmitoylated 3), *Bmf* (*Bcl2*-modifying factor), *Calr* (calreticulin), and *I06Rik* (*E030030I06Rik*). A gene network analysis showed that the genes cluster closely together, and most link to *p53* and *Top1* (Fig. 1B).

To demonstrate that the isolated clones were authentic cheaters, we tested their performance in a developmental context in vivo using ESCs that expressed green fluorescent protein (GFP)-tagged knockdown versions of the top cheater mutants (fig. S4A and fig. S5A) (*shPtafr*, *shp53*, *shTop1*, *shOlfr1387*, *shOlfr305*, *shDcb1*, *shCasp8*, *shMpp3*, *shBmf*, *shCalr*, and *shI06Rik*) and a nontarget control (NTC). We also created a mock cell line containing the empty vector. In each

Fig. 2. In vivo analysis of cheater candidates.

(A) Schematic illustration of the in vivo validation strategy (see text). (B) The contribution of mutant cells to embryonic tissue (somatic cells, squares) and germ cells (GC's, circles) at E14.5 relative to mock cells as determined by PCR. The data represent mean $\pm$ standard error. Ctrl, control; E, embryonic; ES, embryonic stem cells.

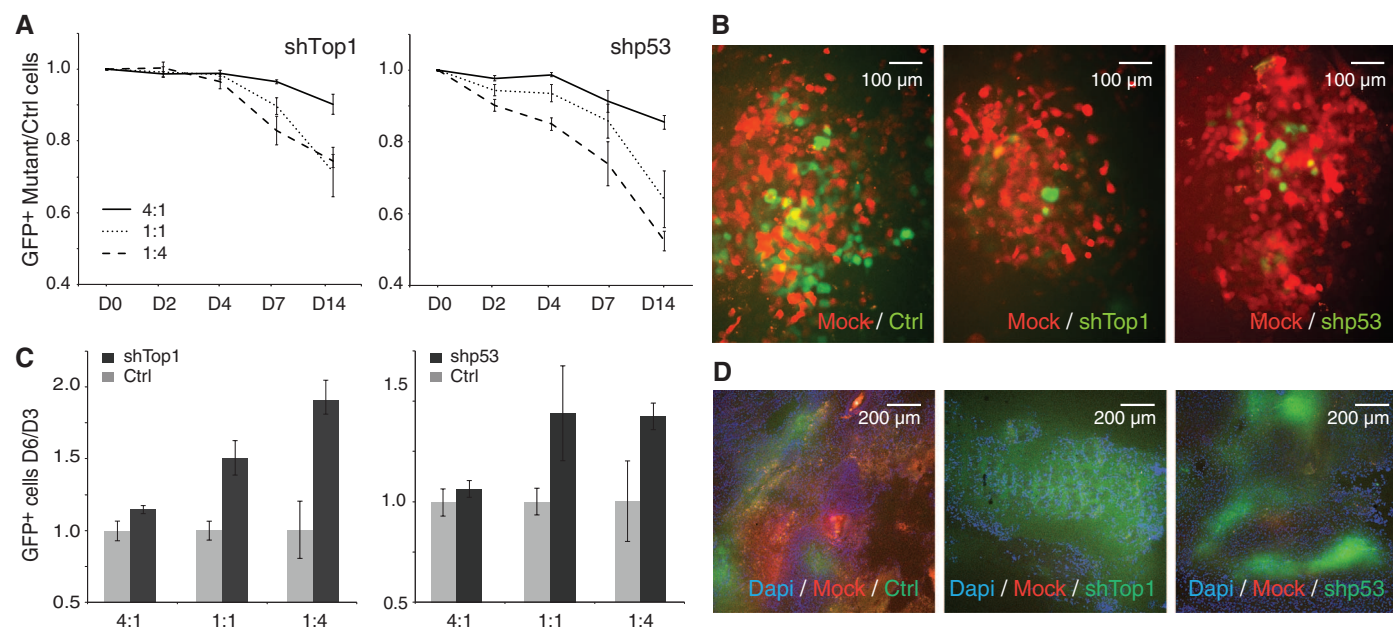
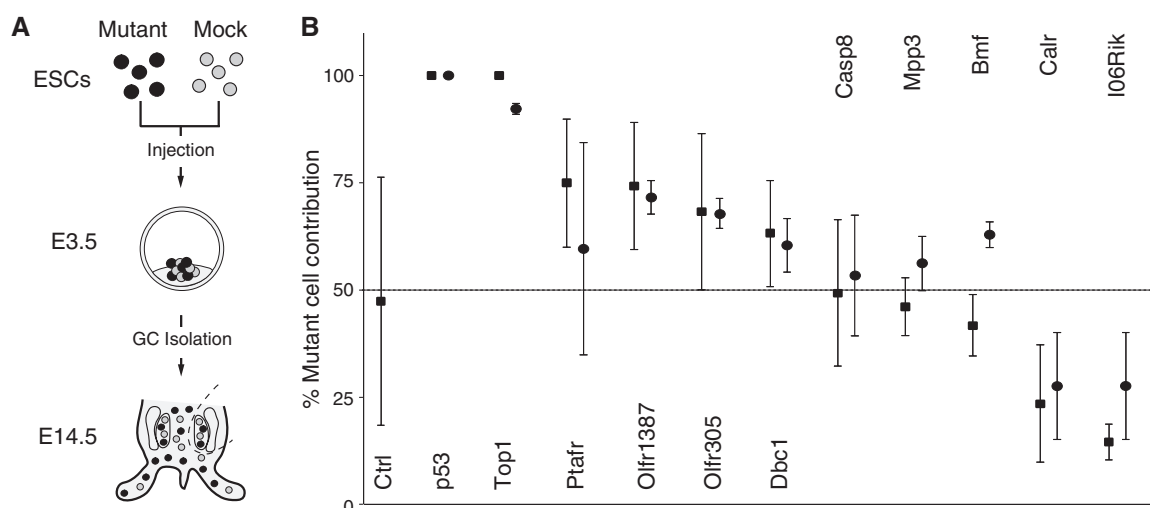


Fig. 3. In vitro behavior of pluripotent and differentiating cheater mutants. (A) Proliferation assay: Coculture of undifferentiated GFP-labeled mutant and RFP-labeled mock cells over the course of 14 days with different starting ratios of mutant:mock, as indicated (means $\pm$ SD). (B) Immunofluorescence images showing mutant and mock ESCs after 10 days of coculture as shown in (A) with an initial ratio of 1:4. (C) Differentiation

assay: Mutant and mock cells were mixed as in (A), and differentiation was induced by EB formation in the absence of Lif (means $\pm$ SD). (D) Teratoma assay: Immunofluorescence analysis of sections from a teratoma grown from a 1:1 mixture injected into hindlegs of severe combined immunodeficiency mice indicates that knockdown cells contributed substantially more to the tumor tissue.

experiment, we injected five tester (provisional cheater mutant) cells and five mock cells into individual embryonic day (E) 3.5 (E3.5) blastocysts, implanted the embryos into pseudopregnant mice, harvested somatic embryonic tissue and germ cells at E14.5, and determined the relative contributions of mutant versus mock cells (Fig. 2A).

The effectiveness of cheating behavior in vivo can be grouped into four classes (Fig. 2B). *p53* or *Top1* knockdown cells (group 1) completely (100%) superseded control cells. It was remarkable that the E14.5 embryos and those that went to term were viable and appeared normal. A certain percentage of *p53* knockout embryos are known to develop defects in neural tube closure (20, 21), whereas *Top1* knockout embryos are not viable. (22). Because our cells regained some *Top1* function at E14.5, it is possible that the competitive phenotype arises from strong knockdown early on (so that later silencing of the retroviral construct is not relevant) or that only mild knockdown is sufficient for the phenotype (fig. S5B). The contribution of group 2 cheater clones (*Ptafr*, *Olf1387*, *Olf1305*, and *Dbc1*) to the embryo was less striking but still significant (60 to 75%). Group 3 (*Casp8*, *Mpp3*, and *Bmf*) clones showed no evidence of competitive behavior (~50%), and group 4 (*Calr* and *106Rik*) clones contributed little (20%) to either the embryo or germ cells. It thus appears likely that mechanisms ensuring cooperation during development are more varied and stringent in vivo than in vitro.

To gain insight into the mechanistic basis for these outcomes, we recapitulated each step of the initial screen using the group 1 genes, beginning with proliferation. Thus, GFP-labeled *p53* or *Top1*

knockdown or control cells were mixed with red fluorescent protein (RFP)-labeled mock cells at three different ratios (1:4, 1:1, and 4:1) and allowed to proliferate for a given time, and their relative contributions were determined (Fig. 3, A and B). This approach not only enabled us to capture the “cheating” cells for subsequent profiling studies (see below), but also allowed us to determine concentration-dependent effects, because homogeneous populations of *p53* and *Top1* mutants exhibited normal growth (fig. S6). This lack of a growth advantage indicates that rare escape mutants were not a major factor in our screening results. To our surprise, both *p53* and *Top1* mutants underperformed when grown in undifferentiated conditions (Fig. 3, A and B), which may suggest that the loss of *p53* or *Top1* activity by stem cells is the signal triggering removal of such defective cells from culture, reminiscent of the “cell competition” described in *D. melanogaster* (10).

Next, we tested the performance of *p53* or *Top1* knockdown cells under differentiation conditions, first in the context of EB development. Here, the outcome was in agreement with the results of the embryological studies presented earlier: The mutant cells expanded disproportionately when mixed with control cells (Fig. 3C). The magnitude of the effect was concentration-dependent, as in the proliferation assay, but in the opposite direction: here, the fewer the cheater cells, the greater their growth advantage. To corroborate our finding using a second differentiation system, we subjected a combination of mutant and control cells to teratoma differentiation conditions. A histologic ex-

amination of the resultant tumors revealed that *p53* and *Top1* knockdown cells contributed substantially more than wild-type cells to all three germ layers (Fig. 3D and fig. S7).

To elucidate the genetic framework that enabled the knockdown cells to predominate, we performed microarray expression analysis (fig. S8A). In these experiments, we mixed knockdown and mock ESCs and collected mRNA on day 4 of coculture, while cells were in the growth phase. These conditions allowed direct comparisons within a single cell type (ESCs) rather than a mixture of different cell types, as seen under differentiation conditions. To extract information specific to cell cooperation as opposed to canonical *p53* and *Top1* functions, we compared results obtained from our mixed (1:4) culture with those obtained after mixing mRNA (also 1:4) from *p53* or *Top1* knockdown cells and mock cells that were grown homotypically. These experiments showed that the cheating phenotype was firmly coupled to the gene network identified by our initial screen and further revealed that the gene signature consisted of a very similar set of genes or gene groups that included *Myc*, *Cdk6*, *Brachyury* (T), and *Jun* (Fig. 4 and fig. S8B). These signatures are closely linked to apoptosis and differentiation and resemble a signature previously linked to cell competition among hematopoietic stem cells (HSCs) (23) (fig. S9 and tables S3 and S4). Although the cells retained many defining characteristics of ESCs, including pluripotency and the ability to participate in three-germ-layer differentiation (as shown in our in vivo studies), it appears that the knockdown of *p53* or *Top1* prompted cells to co-opt a different gene program that facilitated

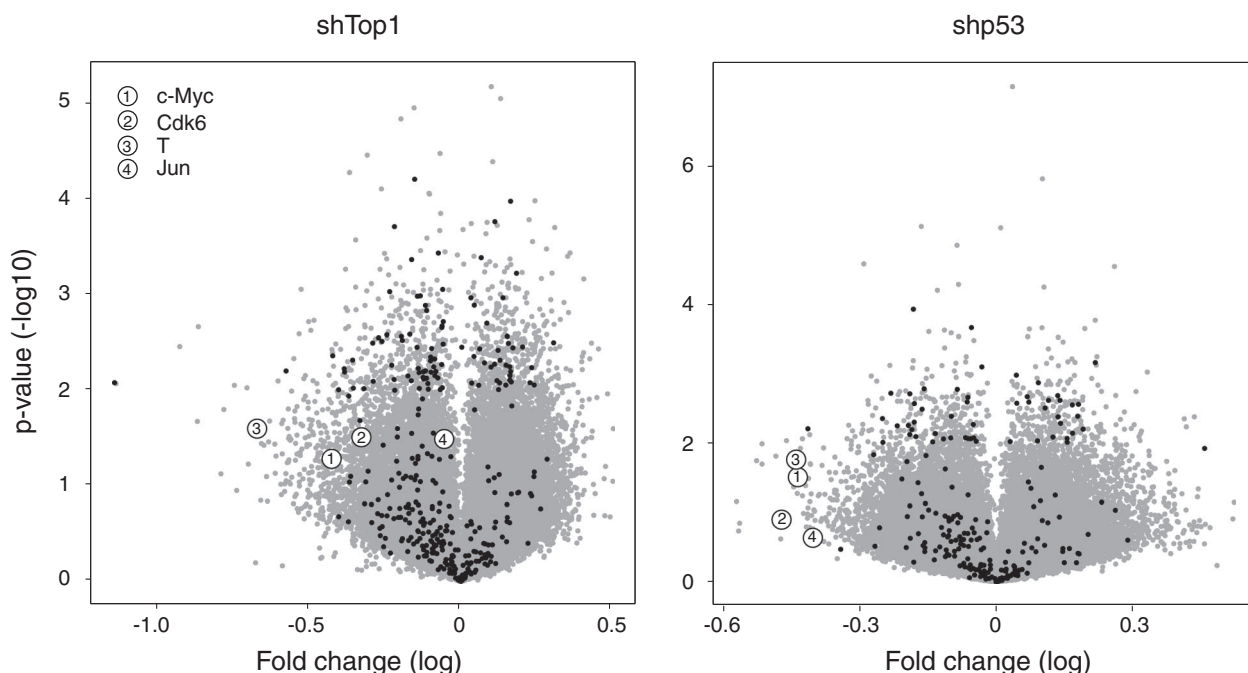


Fig. 4. Volcano plot analyses of microarray results. Black dots represent genes that belong to the Gene Set “GRAESSMANN APOPTOSIS BY DOXORUBICIN D” (tables S3 and S4) which is one of the top gene sets that are significantly enriched when mock cells are cocultured with *shTop1* ($P < 1 \times 10^{-16}$) or *shp53* ($P = 1.26 \times 10^{-9}$) knockdown cells.

more effective colonization of the embryonic niches available during early development.

The behavior of these cheater mutants points to the existence of genetic mechanisms that protect the ability of embryonic cells to cooperate. It is worth mentioning that several of the genes that responded to *p53* or *Top1* knockdown in our studies (*Myc*, *Jun*, and *Dbc1*) have been associated with cell competition in *D. melanogaster* (11–13). It is particularly noteworthy that in the context of DNA damage, murine HSCs with lower levels of p53 outcompete cells with higher levels of the protein (23, 24). We found the opposite in ESCs, and only under differentiation conditions did cells with low levels of p53 (or Top1) outcompete wild-type cells. This could indicate a fundamental difference between ESCs and HSCs, or perhaps the influence of genotoxic stress in the earlier studies; it is also possible that in our experiments, survival of wild-type ESCs under undifferentiated conditions could have been promoted by p53-induced autophagy (25). Although one of the oldest known functions of p53, evident even in the sea anemone (26), is its role in the DNA damage-response pathway, our findings suggest that p53 serves a still more ancient function in enabling cells to respond to signals from other cells to coordinate their activities. Thus, the identification of olfactory receptors by our screen is not surprising, as the ability to cooperate with other cells must entail the ability to sense subtle changes in the microenvironment (indeed, chemotaxis proved to be an important component of the cooperative gene network in our analysis); this result is also in line with the so-called area-code hypothesis (27, 28).

Biological competition has been defined explicitly in terms of a contest between “winners” and “losers,” but the *Drosophila* studies established that cells communicate their growth or metabolic status, guiding each other’s proliferation rate and determining survival (10). Cooperation need not be anthropomorphized to mean anything beyond coordinated activity; such functional interactions can be deemed selfish or altruistic, positive or negative, only in reference to some specific goal. What is surprising in the present study is that mutant cells were not only able to supersede wild-type cells but did so without affecting normal development.

More broadly, our studies place mechanisms of cell coordination during mammalian development in the larger matrix of evolutionary cooperation by identifying genes encoding several of the most ancient, yet still incompletely understood, proteins: p53, Top1, and olfactory receptors. Understanding how cells coordinate their functions might enable us to devise novel strategies for manipulating pluripotent stem cells in vitro (including reprogramming and transdifferentiation to alternative fates) and should illuminate other processes related to tissue homeostasis, including stem cell turnover (as in the skin and immune system) and oncogenesis.

References and Notes

1. A. Rokas, *Annu. Rev. Genet.* **42**, 235–251 (2008).
2. R. E. Michod, *Proc. Natl. Acad. Sci. U.S.A.* **104** (suppl. 1), 8613–8618 (2007).
3. T. Pfeiffer, S. Bonhoeffer, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1095–1098 (2003).
4. A. Khare, G. Shaulsky, *Nat. Rev. Genet.* **7**, 577–584 (2006).
5. A. Sánchez Alvarado, H. Kang, *Exp. Cell Res.* **306**, 299–308 (2005).
6. K. R. Foster, G. Shaulsky, J. E. Strassmann, D. C. Queller, C. R. L. Thompson, *Nature* **431**, 693–696 (2004).
7. L. A. Santorelli et al., *Nature* **451**, 1107–1110 (2008).
8. F. Fiegna, Y.-T. N. Yu, S. V. Kadam, G. J. Velicer, *Nature* **441**, 310–314 (2006).
9. D. C. Hagen, A. P. Bretscher, D. Kaiser, *Dev. Biol.* **64**, 284–296 (1978).
10. L. A. Johnston, *Science* **324**, 1679–1682 (2009).
11. G. Morata, P. Ripoll, *Dev. Biol.* **42**, 211–221 (1975).
12. E. Moreno, K. Basler, *Cell* **117**, 117–129 (2004).
13. C. de la Cova, M. Abril, P. Bellosta, P. Gallant, L. A. Johnston, *Cell* **117**, 107–116 (2004).
14. M. Stadtfeld et al., *Nature* **465**, 175–181 (2010).
15. J. H. Hanna, K. Saha, R. Jaenisch, *Cell* **143**, 508–525 (2010).
16. M. J. Evans, M. H. Kaufman, *Nature* **292**, 154–156 (1981).
17. G. R. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7634–7638 (1981).
18. G. M. Keller, *Curr. Opin. Cell Biol.* **7**, 862–869 (1995).
19. K. Takahashi, S. Yamanaka, *Cell* **126**, 663–676 (2006).
20. J. F. Armstrong, M. H. Kaufman, D. J. Harrison, A. R. Clarke, *Curr. Biol.* **5**, 931–936 (1995).
21. V. P. Sah et al., *Nat. Genet.* **10**, 175–180 (1995).
22. S. G. Morham, K. D. Kluckman, N. Voulomanos, O. Smithies, *Mol. Cell. Biol.* **16**, 6804–6809 (1996).
23. T. Bondar, R. Medzhitov, *Cell Stem Cell* **6**, 309–322 (2010).
24. A. Marusyk, C. C. Porter, V. Zaberezhnyy, J. DeGregori, *PLoS Biol.* **8**, e1000324 (2010).
25. D. Crighton et al., *Cell* **126**, 121–134 (2006).
26. S. Pankow, C. Bamberger, *PLoS ONE* **2**, e782 (2007).
27. W. J. Dreyer, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9072–9077 (1998).
28. C. D. Buckley et al., *Cell. Immunol.* **236**, 29–41 (2005).

Acknowledgments: We thank G. Shaulsky and J. Gilbert for helpful discussions and experimental suggestions. We thank K. Hochedlinger for providing the Oct4-GFP iPSCs. The work was funded by the Huffington Foundation and by the NIH (grants R01 GM077442 and P01 GM81627). Microarray data have been deposited at Gene Expression Omnibus (GSE49814).

Supplementary Materials

www.sciencemag.org/content/341/6153/1511/suppl/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 to S4
References (29–31)

7 June 2013; accepted 28 August 2013
Published online 12 September 2013;
10.1126/science.1241628

Distinguishable Epidemics of Multidrug-Resistant *Salmonella* Typhimurium DT104 in Different Hosts

A. E. Mather,¹ S. W. J. Reid,^{2†} D. J. Maskell,³ J. Parkhill,¹ M. C. Fookes,¹ S. R. Harris,¹ D. J. Brown,⁴ J. E. Coia,⁴ M. R. Mulvey,⁵ M. W. Gilmour,^{5*} L. Petrovska,⁶ E. de Pinna,⁷ M. Kuroda,⁸ M. Akiba,⁹ H. Izumiya,¹⁰ T. R. Connor,^{1†} M. A. Suchard,¹¹ P. Lemey,¹² D. J. Mellor,¹³ D. T. Haydon,¹³ N. R. Thomson^{1†}

The global epidemic of multidrug-resistant *Salmonella* Typhimurium DT104 provides an important example, both in terms of the agent and its resistance, of a widely disseminated zoonotic pathogen. Here, with an unprecedented national collection of isolates collected contemporaneously from humans and animals and including a sample of internationally derived isolates, we have used whole-genome sequencing to dissect the phylogenetic associations of the bacterium and its antimicrobial resistance genes through the course of an epidemic. Contrary to current tenets supporting a single homogeneous epidemic, we demonstrate that the bacterium and its resistance genes were largely maintained within animal and human populations separately and that there was limited transmission, in either direction. We also show considerable variation in the resistance profiles, in contrast to the largely stable bacterial core genome, which emphasizes the critical importance of integrated genotypic data sets in understanding the ecology of bacterial zoonoses and antimicrobial resistance.

Salmonella enterica subspecies *enterica* is one of the most common bacterial pathogens of humans and other animals (1, 2). The global burden of disease caused by *Salmonella* infections is substantial, with more than 90 million human cases of gastroenteritis alone occurring each year (3). The annual cost of these infections is estimated to be about €3 billion in the European Union (4) and about \$2.7 billion in the United States (5). The public health impact is exacerbated by antimicrobial resistance (AMR), which leads to increased morbidity, mortality, and treatment costs (6, 7). In our study, the interrelated epidemiologies of *Salmonella* and

AMR were examined at the level of the genome. Our results challenge the established view that the human and animal epidemics are synonymous (8–11) and show that the phylogenetic associations both within and between the resistance determinants and the host bacteria are different.

Throughout the 1990s, there was a global epidemic of multidrug-resistant *S. Typhimurium* definitive type 104 (DT104) in animals and humans (12, 13). The DT104 epidemic was important because of its widespread prevalence and perceived zoonotic nature, as well as the high frequency of resistance to a wide range of commonly used antimicrobials, particularly ampicillin,

chloramphenicol, streptomycin, sulfonamides, and tetracycline. The primary source of human DT104 infections is thought to be the animal population, particularly local animals, through both direct contact and food-borne transmission (9, 10, 14). Using whole-genome sequencing, we have studied the course of the epidemic in Scotland, a restricted geographical location that has a well-characterized collection of DT104 bacteria isolated from both humans and other animals over a 22-year period. By interrogating isolates collected from sympatric and contemporaneous hosts and using isolates from geographically diverse hosts from other countries to put them in phylogenetic context, we have an unprecedented opportunity to profile the evolution and spread of a local epidemic of this globally important zoonotic pathogen.

To investigate the evolutionary changes that characterize the long-term epidemic and to assess the roles of animals and humans in the spread of both the bacterium and AMR, we sequenced 142 isolates from humans and 120 isolates from other animals in Scotland sampled from 1990 to 2011. To set the Scottish isolates within a broader international context, 111 isolates were included from other locations around the world (table S1). Sequence reads were mapped against our reference chromosome of DT104 and its virulence plasmid, and genomes were assembled de novo to identify unique regions (15). Maximum likelihood methods were used to produce a phylogenetic tree based on single-nucleotide polymorphisms (SNPs) in the core genome (15). In this tree, we identified a main clade of related DT104 isolates (29 to 123 SNPs compared with the reference core genome) and a separate subset of 14 atypical isolates (620 to 709 SNPs compared with the reference core genome) (fig. S1). Within the main clade, there were 2765 variable sites across the 359 isolates, with pairwise differences between

isolates ranging from 0 to 167 SNPs (Fig. 1). Most SNPs were sporadically distributed in the core genome throughout the phylogenetic tree, with relatively few genes showing nonsynonymous SNPs in >5 isolates (fig. S2). These data indicate that the rate of change within the core genome over the 22-year epidemic period, 3.4×10^{-7} substitutions per site per year [95% highest posterior density (HPD) interval: 3.1×10^{-7} to 3.7×10^{-7}], is in line with that seen in other *S. Typhimurium* (16). Although confirmed phenotypically as DT104 by standard phage typing, the 14 atypical isolates are more similar genetically to the non-DT104 *S. Typhimurium* reference strain LT2, which demonstrates the lack of resolution of traditional typing techniques. Isolates from all geographical locations can be found near the root of the tree, consistent with the rapid global spread of DT104 (12). However, many of these isolates, particularly those from England and Wales, can also be found interspersed with Scottish isolates throughout the rest of the tree. The most deeply rooted are primarily derived from humans and do not have *Salmonella* genomic island 1 (SGI1), a 43-kb genomic island with a 13-kb multidrug-resistance region (17) that is characteristic of the epidemic strain.

To evaluate interspecies transmission and how DT104 circulated in Scotland, Bayesian phylogenetic diffusion models (18, 19) were used to reconstruct the host population of each branch of the phylogenetic tree (Fig. 2A), using the 135 human and 113 animal Scottish DT104 isolates. The most recent common ancestor of DT104 in Scotland is predicted to date to 1968 (95% HPD interval: 1962 to 1976), consistent with reports of antimicrobial-susceptible DT104 strains iso-

lated from human cases of infection since the 1960s in England and Wales (13), with the first report of multidrug-resistant DT104 in Scotland in the mid 1980s (13). What can be observed in Figs. 1 and 2A is that, although there are isolates from a single host population that cluster together, isolates from animals and humans are also interspersed throughout the tree. However, quantification of the association between phylogeny and host population shows that the clustering is not fully randomized (15). Furthermore, similar to the maximum likelihood tree (Fig. 1), the most deeply rooted branches are estimated to be human (Fig. 2A), but it is difficult to infer with certainty the direction of transmission from these observations alone. Overall, the reconstructed host populations of the branches within the tree suggest that there were relatively few animal-to-human or human-to-animal transitions (Fig. 2B). Using Markov jumps to estimate the number of unobserved human-to-animal and animal-to-human transitions along each branch (15), we show the overall numbers of animal-to-human and human-to-animal transitions were similar, which indicates that the majority of human infections in Scotland were unlikely to have been sourced from the local animal population. Markov rewards, from which inferences may be made with respect to which populations might act as sources and which might act as sinks, showed that, although the human isolates represented just 54% of the sample, 62% (666 years, 95% HPD: 545 to 771) of the total phylogenetic tree length was in the human state (15). Conversely, 38% (400 years, 95% HPD: 318 to 521) of the total tree length was spent in the animal state. Our conclusions are consistent when we perform

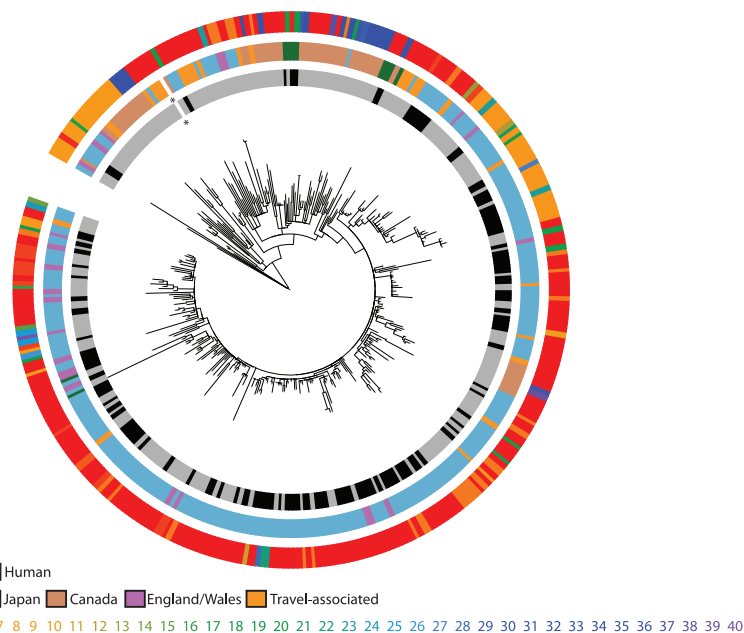


Fig. 1. Phylogeny of Scottish and global *S. Typhimurium* DT104, rooted on *S. Typhimurium* SL1344. Colored rings indicate, from the center out, the host and country of origin, and the unique genotypic AMR profile of each isolate (table S6); the asterisk indicates the location of the reference isolate HF937208. The same tree with bootstrap values added is shown in fig. S5.

¹Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK. ²Royal Veterinary College, North Mymms, Hatfield AL9 7TA, UK. ³Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK. ⁴Scottish *Salmonella Shigella* and *Clostridium difficile* Reference Laboratory, Stobhill Hospital, Glasgow G21 3UW, UK. ⁵National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, R3E 3R2, Canada. ⁶Animal Health and Veterinary Laboratories Agency, Weybridge, KT15 3NB, UK. ⁷Gastrointestinal Bacteria Reference Unit, Public Health England, Colindale, London NW9 5EQ, UK. ⁸Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo 162-8640, Japan. ⁹Bacterial and Parasitic Disease Research Division, National Institute of Animal Health, Ibaraki 305-0856, Japan. ¹⁰Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo 162-8640, Japan. ¹¹Departments of Biomathematics and Human Genetics, David Geffen School of Medicine at UCLA, and Department of Biostatistics, UCLA Fielding School of Public Health, University of California, Los Angeles, CA 90095, USA. ¹²Department of Microbiology and Immunology, Rega Institute, KU Leuven, 3000 Leuven, Belgium. ¹³Boyd Orr Centre for Population and Ecosystem Health, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK.

*Present address: Diagnostic Services Manitoba, Winnipeg, R3A 1R9, Canada.

†Present address: Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK.

‡Corresponding author. E-mail: swjreid@rvc.ac.uk (S.W.J.R.); nrt@sanger.ac.uk (N.R.T.)

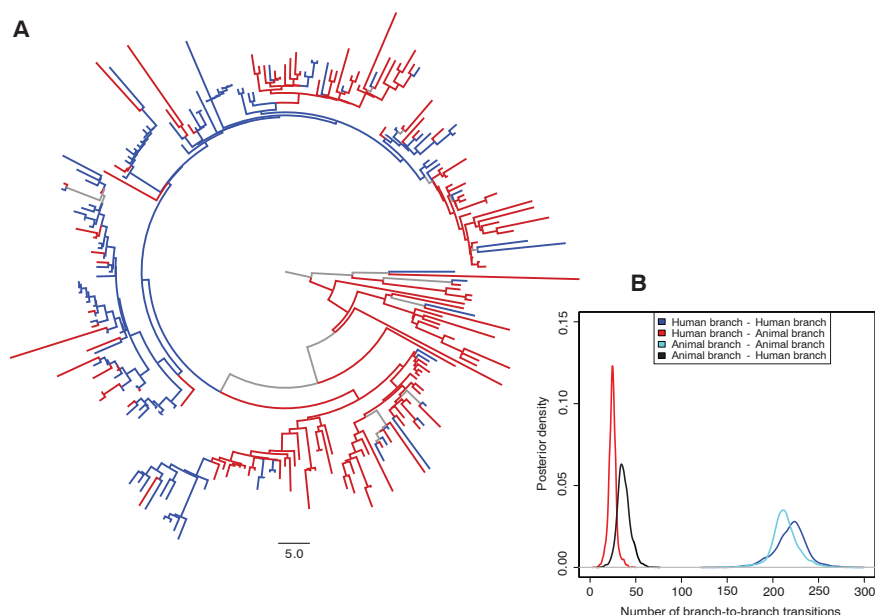


Fig. 2. Bayesian maximum clade credibility phylogenetic tree and most probable ancestral state reconstruction of host population for *S. Typhimurium* DT104 in Scotland. (A) Branches with a reconstructed state (host population) posterior probability of >0.75 are colored red for human, blue for animal; branches with a state probability of <0.75 are colored gray. The same tree is shown in fig. S6 with branch width scaled by the posterior probabilities. (B) Posterior density plot of the numbers of human branches ancestral to human branches, human branches ancestral to animal branches, animal branches ancestral to human branches, and animal branches ancestral to animal branches integrated over the subsample of 3600 phylogenetic trees with reconstructed host population states along branches obtained by using BEAST. These results suggest (i) circulation of DT104 predominantly within animals and humans separately, with only a low frequency of spillover in both directions, and/or (ii) animals and humans were each sinks for different and separate sources of infection, with only a low frequency of spillover in both directions.

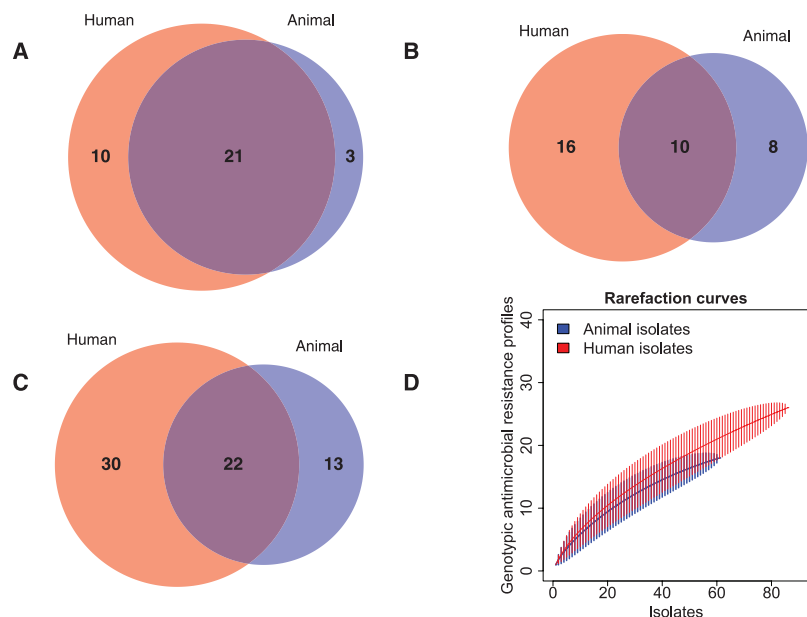


Fig. 3. Venn diagrams demonstrating the degree of overlap in AMR between the human and animal populations of *S. Typhimurium* DT104. (A) The numbers of shared and unique AMR determinants (acquired resistance genes or SNPs known to confer resistance) in the 147 Scottish human and animal DT104 isolates investigated for AMR diversity; (B) the numbers of shared and unique AMR profiles (unique combinations of AMR determinants) in the 147 isolates; (C) the numbers of shared and unique AMR phenotypic profiles (unique combinations of AMR phenotypes) in the original 5200 surveillance isolates of DT104, 1990–2004, Scotland (11). (D) Rarefaction curves, with 95% confidence intervals (vertical lines), of the number of genotypic AMR profiles in the 147 isolates investigated for AMR diversity, demonstrating similar sampling intensity.

these analyses on animal isolates by species and with only cattle isolates, the main animal reservoir (15, 20). These results support two novel hypotheses: (i) DT104 was predominantly circulating separately within each population in Scotland, with a low frequency of spillover in both directions, and/or (ii) that animals and humans were each sinks for a different and separate source of infection, also with a low level of spillover. Scotland is a net importer of food (21); for example, by value, 58% of all red meat and 38% of raw beef are of non-Scottish origin (22). However, given a lack of surveillance data on food, attribution to this potential source cannot be quantified. Given the likely importance of imported food to the disease burden in humans, the greater global mobility of the human population, and the recognition of this as a risk factor for bacterial infections (23, 24), we suggest that, although these two hypotheses are not mutually exclusive, the second scenario is the more likely.

Our results show that the ecological diversity of AMR, at the level of the genome, is greater in the isolates from the human population than in those from animals and is not tightly coupled to the bacterial evolutionary history. Examining the distribution of the phenotypic AMR profiles throughout the molecular phylogenetic tree (fig. S3) (15) allowed us to investigate the degree to which the evolutionary history of the organism is linked to that of the resistance determinants it carries, regardless of whether they are genes or SNPs known to confer resistance. We show that isolates demonstrating the same phenotypic resistance profile, even those putatively conferred by SG11 (17) (fig. S3A), do not cluster together exclusively.

The diversity of AMR was assessed further by interrogating the presence or absence of resistance determinants and profiles in the 147 Scottish isolates selected to cover the range of phenotypic resistance profiles observed in the epidemic period (Fig. 3) (15). A greater number of both resistance determinants and genotypic-resistance profiles are found in the human isolates. As demonstrated by rarefaction analysis (Fig. 3D), these results cannot be accounted for by differential sampling intensity. Multiple, independent recombination events in the multidrug-resistance region occurred, as variants of SG11 (25) are found throughout the tree (fig. S4). Similarly, although there are some clades with a single AMR gene profile, in many instances, profiles can be found in multiple clades and may be due to the gain or loss by horizontal gene transfer of accessory AMR determinants and their plasmid vectors (tables S2 to S5). Although the propensity for horizontal transmission is well recognized (26), these results provide both a unique insight into the genetic flux occurring across a defined 22-year epidemic and a baseline for the concomitant genetic flux in the DT104 core genome. In contrast to the relatively stable core genome with an expected substitution rate, the AMR features of these isolates varied appreciably throughout the time period.

Most transmission of DT104 has hitherto been considered to be from animals to humans (27–29), but this view has been based primarily on outbreak investigations. However, on the basis of whole-genome sequence analysis, we provide a historical perspective on the epidemic that leads to a different inference. This is the largest study of its kind, utilizing tools with the finest resolution available to investigate the associations of *Salmonella* Typhimurium DT104 and AMR between and within colocated human and animal populations, and has enabled us to address two separate issues with this data set, those of the origins and dissemination of AMR and also of *Salmonella*. First, the greater diversity of AMR genes and profiles in the human DT104 isolates suggests that there were other sources contributing to the diversity of the human resistance burden than the local animals; it is noteworthy that this analysis of molecular data leads to identical conclusions to those based on phenotype (11). Second, we show that a large proportion of transmission of the bacterium appears to have occurred within each host population, and/or that there were separate sources of DT104 for the humans and local animals, with a small degree of spillover in both directions. If the majority of human infections were obtained from locally produced food or direct contact with animals, the human isolates should be a subset of the animal isolates, but we do not observe this.

With humans having a more diverse source of infections than the local animal population, it is probable that imported food, foreign travel, and environmental reservoirs are significant sources of *Salmonella* and AMR in humans in our study (23, 30), but surveillance data simply do not exist in any coherent form locally, nor consistently across the international community, to quantify the importance of these different sources. If we are to address this deficit, there is an urgent need to construct relevant, robust, and harmonized surveillance protocols to capture this information. Similarly, although the Scottish isolates are similar to those from other countries, this investigation needs to be conducted in other organisms and environments to determine the generality of our conclusions across different agricultural, political, and sociological conditions. This study challenges current views on the contribution of the local animal reservoir as a source of *Salmonella* and AMR in humans. It demonstrates the critical importance of acquiring targeted genotypic data sets integrated across the veterinary and public health sectors in order to understand the ecology of bacterial zoonoses, identify the sources of AMR, and formulate responsible evidence-based control strategies.

References and Notes

1. Herikstad, Y., Motarjemi, R. V. Tauxe, *Epidemiol. Infect.* **129**, 1–8 (2002).
2. T. S. Wallis, P. A. Barrow, in *EcoSal—Escherichia coli and Salmonella: Cellular and Molecular Biology*, G. Dougan, Ed. (ASM Press, Washington, DC, 2005).
3. S. E. Majowicz et al., *Clin. Infect. Dis.* **50**, 882–889 (2010).

4. European Food Safety Authority (EFSA), *Salmonella* (2011); www.efsa.europa.eu/en/topics/topic/salmonella.htm.
5. U.S. Department of Agriculture, Foodborne Illness Cost Calculator (2011); <http://webarchives.cdlib.org/sw1rf5mh0k/http://www.ers.usda.gov/Data/FoodborneIllness/>.
6. J. J. Engemann et al., *Clin. Infect. Dis.* **36**, 592–598 (2003).
7. S. C. Davies, “Annual Report of the Chief Medical Officer; volume two, 2011: Infections and the rise of antimicrobial resistance” (Department of Health, London, 2013).
8. M. K. Glynn et al., *N. Engl. J. Med.* **338**, 1333–1339 (1998).
9. P. G. Wall et al., *Vet. Rec.* **136**, 591–592 (1995).
10. A. M. Dechet et al., *Clin. Infect. Dis.* **42**, 747–752 (2006).
11. A. E. Mather et al., *Proc. Biol. Sci.* **279**, 1630–1639 (2012).
12. M. Helms, S. Ethelberg, K. Mølbak, *Emerg. Infect. Dis.* **11**, 859–867 (2005).
13. E. J. Threlfall, *J. Antimicrob. Chemother.* **46**, 7–10 (2000).
14. T. E. Besser et al., *Epidemiol. Infect.* **124**, 193–200 (2000).
15. Materials and methods are available as supplementary materials on Science Online.
16. C. K. Okoro et al., *Nat. Genet.* **44**, 1215–1221 (2012).
17. D. Boyd et al., *J. Bacteriol.* **183**, 5725–5732 (2001).
18. A. J. Drummond, M. A. Suchard, D. Xie, A. Rambaut, *Mol. Biol. Evol.* **29**, 1969–1973 (2012).
19. P. Lemey, A. Rambaut, A. J. Drummond, M. A. Suchard, *PLoS Comput. Biol.* **5**, e1000520 (2009).
20. N. Calvert, W. C. Stewart, W. J. Reilly, *Vet. Rec.* **143**, 351–354 (1998).
21. “Food and drink in Scotland: Key facts 2012” (Scottish Government, Edinburgh, 2012); www.scotland.gov.uk/Resource/0038/00389160.pdf.
22. C. Revoredo-Giha, W. Wrieden, B. Kupiec-Teahan, P. Leat, A. Fearne, L. Cacciolatti, “Analysis of red and processed meat purchases in Scotland using representative supermarket panel data” (Food Standards Agency in Scotland, Aberdeen, 2009); www.foodbase.org.uk/admintools/reportdocuments/338-1-594_S14046.pdf.
23. M. A. Davis, D. D. Hancock, T. E. Besser, *J. Lab. Clin. Med.* **140**, 135–141 (2002).
24. L. Skjott-Rasmussen et al., *Int. J. Food Microbiol.* **131**, 277–279 (2009).

25. M. R. Mulvey, D. A. Boyd, A. B. Olson, B. Doublet, A. Cloeckert, *Microbes Infect.* **8**, 1915–1922 (2006).
26. A. O. Summers, *Anim. Biotechnol.* **17**, 125–135 (2006).
27. K. Mølbak et al., *N. Engl. J. Med.* **341**, 1420–1425 (1999).
28. D. L. Fone, R. M. Barker, *Commun. Dis. Rep. CDR Rev.* **4**, R136–R140 (1994).
29. F. J. Angulo, K. R. Johnson, R. V. Tauxe, M. L. Cohen, *Microb. Drug Resist.* **6**, 77–83 (2000).
30. S. Zhao et al., *J. Food Prot.* **69**, 500–507 (2006).

Acknowledgments: A.E.M. was funded by the William Stewart Fellowship while at the University of Glasgow. A.E.M., S.R.H., M.C.F., T.R.C., J.P., and N.R.T. are supported by Wellcome Trust grant 098051. P.L. and M.A.S. acknowledge funding from the European Union Seventh Framework Programme (FP7/2007-2013) under European Research Council Grant agreement no. 260864. M.A.S. is supported in part by NIH R01 grants AI07034 and HG006139 and NSF grant DMS-1264153. The authors would like to thank J. Cotton (Wellcome Trust Sanger Institute) for helpful discussions and assistance. Genome sequences are deposited with the European Nucleotide Archive (study accessions ERP000244, ERP000270, ERP000994) and DNA Data Bank of Japan (accession DRA000942). The finished chromosome and plasmid for *Salmonella* Typhimurium DT104 are submitted to the European Molecular Biology Laboratory, accessions HF937208 and HF937209, respectively.

Supplementary Materials

www.sciencemag.org/content/341/6153/1514/suppl/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 to S12
References (31–70)

15 May 2013; accepted 16 August 2013
Published online 12 September 2013;
10.1126/science.1240578

The Inhibitory Circuit Architecture of the Lateral Hypothalamus Orchestrates Feeding

Joshua H. Jennings,^{1,2} Giorgio Rizzi,^{1,3} Alice M. Stamatakis,^{1,2}
Randall L. Ung,¹ Garret D. Stuber^{1,2,4,5,6*}

The growing prevalence of overeating disorders is a key contributor to the worldwide obesity epidemic. Dysfunction of particular neural circuits may trigger deviations from adaptive feeding behaviors. The lateral hypothalamus (LH) is a crucial neural substrate for motivated behavior, including feeding, but the precise functional neurocircuitry that controls LH neuronal activity to engage feeding has not been defined. We observed that inhibitory synaptic inputs from the extended amygdala preferentially innervate and suppress the activity of LH glutamatergic neurons to control food intake. These findings help explain how dysregulated activity at a number of unique nodes can result in a cascading failure within a defined brain network to produce maladaptive feeding.

More than half a century ago, experiments in rodents and other species revealed that gross neuroanatomical manipulations of the lateral hypothalamus (LH) alter diverse behaviors, including feeding (1–3). Although direct anatomical and neuropharmacological manipulations (4, 5) within the LH produce profound alterations in a variety of motivated behaviors, they provide limited mechanistic insight into the discrete circuit connections within the LH

that regulate precise behaviors such as feeding. Given the circuit complexity within the LH (6), we aimed to dissect the neurocircuitry between the LH and a principal afferent from the extended amygdala.

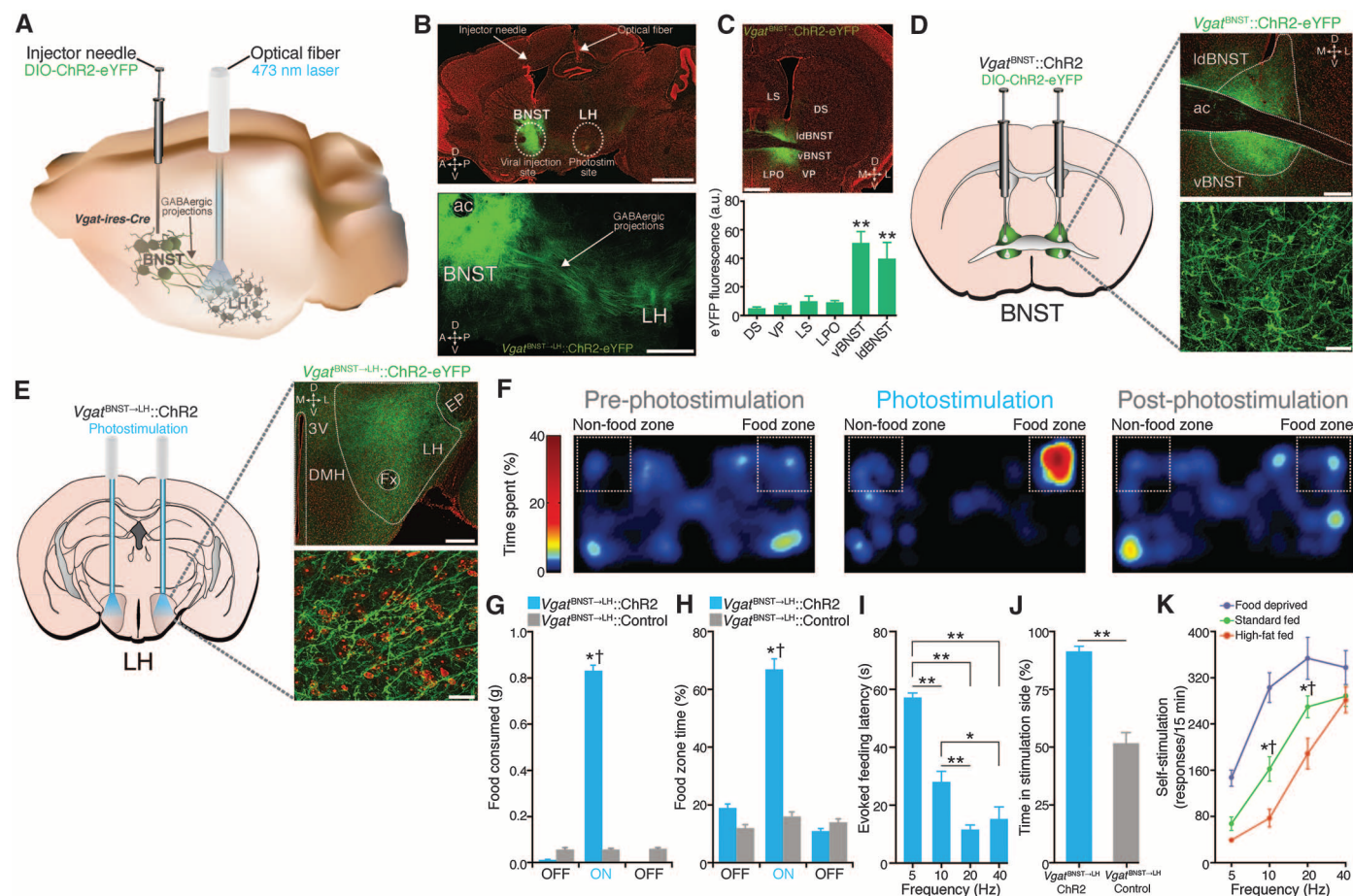
The bed nucleus of the stria terminalis (BNST), a neural component of the extended amygdala (7), is a key integrator of diverse motivational states through its interactions with various synaptic targets, including the ventral tegmental area

(VTA) (8) and the LH (9). The BNST is composed primarily of γ -aminobutyric acid–releasing (GABAergic) cells (10), and consumption of

food activates BNST neurons (11). Therefore, we considered the BNST and its inhibitory projections to the LH as an important candidate for regulating feeding. We targeted a Cre-inducible viral construct coding for Channelrhodopsin-2 fused to enhanced yellow fluorescent protein (ChR2-eYFP) into the BNST of *Vgat-ires-Cre* mice and positioned optical fibers above the LH for in vivo photostimulation of *Vgat*^{BNST→LH} projection fibers (Fig. 1, A to E, and fig. S1). Optogenetic activation of this inhibitory pathway rapidly produced voracious feeding behavior in well-fed mice (Fig. 1, F to I, fig. S2, and movie S1). We explored the motivational valence of this pathway by testing mice in real-time place preference and self-stimulation assays (see supplementary materials).

Vgat^{BNST→LH::ChR2} mice exhibited a significant place preference for a photostimulation-paired chamber (Fig. 1J and fig. S2) and actively nose-poked for photoactivation of the circuit. Food deprivation significantly augmented, whereas satiety significantly attenuated, *Vgat*^{BNST→LH::ChR2} self-stimulation (Fig. 1K and fig. S2). The evoked feeding response was specific to the *Vgat*^{BNST→LH} pathway. Photoactivation of *Vgat*^{BNST→VTA} projections did not elicit feeding behavior (figs. S3 and S4).

Because high-caloric diets can facilitate overeating (12), we determined whether consumption induced by *Vgat*^{BNST→LH} circuit activation was directed toward palatable energy-dense foods (see supplementary materials). Well-fed



Photostimulation of *Vgat*^{BNST→LH} projections significantly increased grain-based (standard) food intake ($F_{2,24} = 201.6$, $P < 0.001$) (G) and food zone time ($F_{2,24} = 18.61$, $P < 0.001$, $n = 5$ mice per group) (H). (I) Higher photostimulation frequencies significantly decreased evoked feeding latencies ($F_{3,56} = 48.89$, $P < 0.001$, $n = 9$ mice). (J) *Vgat*^{BNST→LH::ChR2} mice spent significantly more time in the photostimulation-paired side compared with controls ($P < 0.001$, $n = 5$ mice per group). (K) Food-deprived *Vgat*^{BNST→LH::ChR2} mice nose-poked significantly more for 10- and 20-Hz photostimulation when compared with 2 days of standard-fed ad libitum and to 2 days of standard-fed ad libitum supplemented with 2 hours of high-fat food exposure before the self-stimulation session ($F_{2,204} = 40.87$, $P < 0.001$, $n = 9$ mice). All values for all figures represent mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.001$ (Student's t test or analysis of variance followed by Bonferroni post hoc comparisons, where applicable). Dagger symbol denotes significance compared to all manipulations.

food activates BNST neurons (11). Therefore, we considered the BNST and its inhibitory projections to the LH as an important candidate for regulating feeding. We targeted a Cre-inducible viral construct coding for Channelrhodopsin-2 fused to enhanced yellow fluorescent protein (ChR2-eYFP) into the BNST of *Vgat-ires-Cre* mice and positioned optical fibers above the LH for in vivo photostimulation of *Vgat*^{BNST→LH} projection fibers (Fig. 1, A to E, and fig. S1). Optogenetic activation of this inhibitory pathway rapidly produced voracious feeding behavior in well-fed mice (Fig. 1, F to I, fig. S2, and movie S1). We explored the motivational valence of this pathway by testing mice in real-time place preference and self-stimulation assays (see supplementary materials).

$Vgat^{BNST \rightarrow LH}::ChR2$ mice showed a strong preference for high-fat food during photostimulation exposure (table S1 and movie S1), suggesting that activation of the $Vgat^{BNST \rightarrow LH}$ circuit is sufficient for eliciting feeding that is preferential for calorie-dense substances even when energy requirements are satisfied.

To examine whether endogenous activity of the $Vgat^{BNST \rightarrow LH}$ pathway is important for feeding, we transduced BNST-GABAergic neurons (Fig. 2A) and their axons that innervate the LH (Fig. 2B and fig. S5) with the inhibitory opsin, archaerhodopsin (eArch3.0-eYFP) (13, 14). Suppression of presynaptic BNST-GABAergic signaling through eArch3.0 activation resulted in enhanced LH-postsynaptic neuronal activity during anesthetized extracellular recordings (Fig. 2,

C to E). Despite the influence of hunger, photoinhibition of the $Vgat^{BNST \rightarrow LH}$ circuit reduced feeding in food-deprived mice (Fig. 2, F to J, and fig. S6). Furthermore, photoinhibition of $Vgat^{BNST \rightarrow LH}$ projections led to a significant avoidance of a photoinhibition-paired chamber (Fig. 2K and fig. S6).

The hypothalamus contains numerous genetically distinct neuronal populations (15–21). We thus characterized the molecular phenotype of the postsynaptic LH neuronal targets that receive functional $Vgat^{BNST \rightarrow LH}$ innervation. We paired photostimulation of $Vgat^{BNST \rightarrow LH}$ inputs with whole-cell recordings in conjunction with multiplexed gene expression profiling of individual LH neurons in brain slices (Fig. 3A and supplementary materials). We focused on a set

of genes known to be heterogeneously expressed in the LH and whose products have been implicated in feeding (22). BNST-GABAergic inputs formed strong functional connections with postsynaptic LH neurons that expressed significantly higher levels of *Vglut2*. In contrast, weakly innervated LH neurons displayed significantly lower levels of *Vglut2* and higher levels of *Vgat* expression (Fig. 3, B and C, fig. S7, and table S2).

We confirmed these findings by using modified rabies virus tracing techniques to identify the monosynaptic inputs to glutamatergic and GABAergic neurons in the LH (Fig. 3D). In *Vglut2-ires-Cre* and *Vgat-ires-Cre* mice, we targeted Cre-inducible avian sarcoma leucosis virus glycoprotein EnvA receptor (TVA) (AAV5-FLEX-TVA-mCherry) and rabies virus envelope glyco-

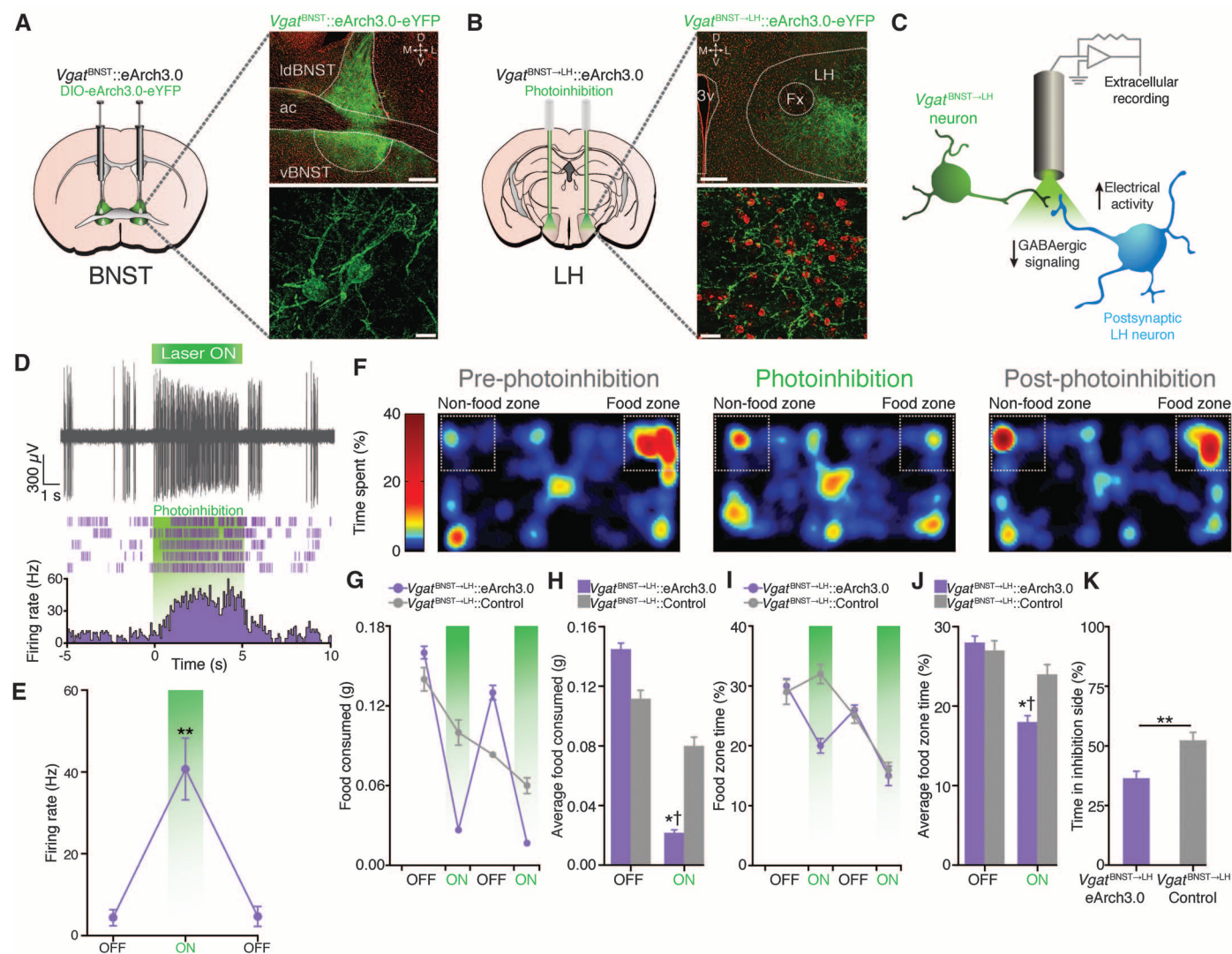
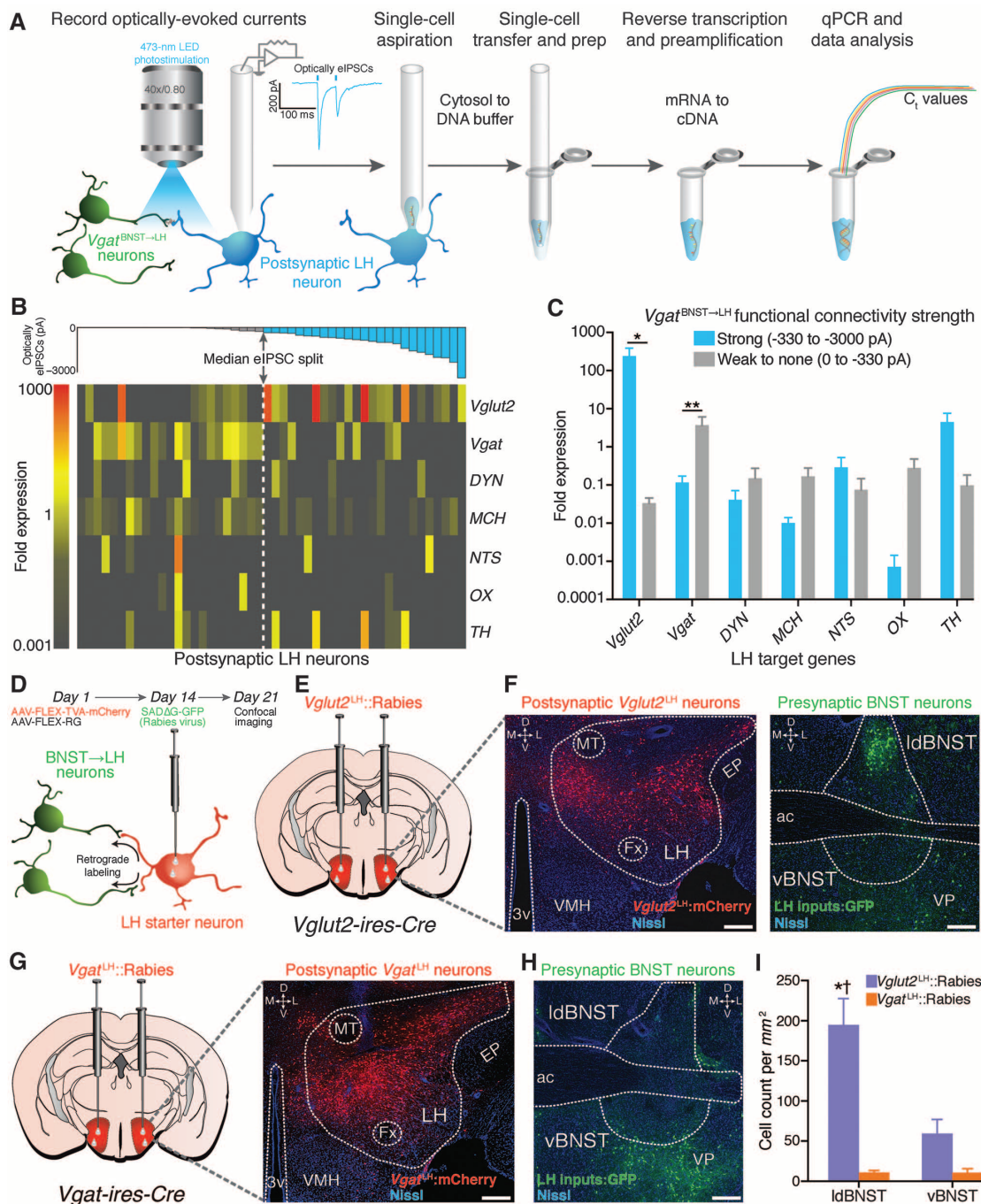


Fig. 2. $Vgat^{BNST \rightarrow LH}$ circuit inhibition diminishes feeding in food-deprived mice and is aversive. (A and B) eArch3.0-eYFP expression in the BNST (A) and axonal projections in the LH (B) in $Vgat^{BNST \rightarrow LH}$ mice. Scale bars, 200 μ m (top), 20 μ m (bottom). (C) Schematic for anesthetized in vivo extracellular recordings in the LH. (D) Example trace from a single LH unit (top) and its representative peri-event histogram and raster (bottom). (E) The average firing rate of light-responsive LH units significantly increased during the 5-s photoinhibition trials

($F_{2,12} = 19.52$, $P < 0.001$, $n = 5$ units from $n = 3$ mice). (F) Spatial location heat maps in 10-min epochs before, during, and after photoinhibition. (G and H) Photoinhibition of $Vgat^{BNST \rightarrow LH}$ projections significantly decreased standard food intake ($F_{1,44} = 16.30$, $P < 0.001$) and time spent in the food zone (I and J) ($F_{1,44} = 2.43$, $P = 0.028$, $n = 6$ mice per group). (K) $Vgat^{BNST \rightarrow LH}::eArch3.0$ mice spent significantly less time in the photoinhibition-paired side when compared with controls ($P = 0.004$, $n = 6$ mice per group).

Fig. 3. *Vgat*^{BNST→LH} projections preferentially target LH glutamatergic neurons.

(A) Schematic for Chr2-assisted circuit mapping with single-cell gene expression profiling. (B) Color-coded fold expression of all target genes from all recorded LH neurons. *Vglut2*, vesicular glutamate transporter-2; *Vgat*, vesicular GABA transporter; *DYN*, dynorphin; *MCH*, melanin-concentrating hormone; *NTS*, neurotensin; *OX*, orexin/hypocretin; *TH*, tyrosine hydroxylase. (C) The average fold expression for *Vglut2* was significantly higher in postsynaptic LH neurons that display large optically evoked inhibitory postsynaptic current amplitudes (strongly innervated) compared with weakly innervated LH neurons ($U = 169.0$, $P = 0.016$, $n = 6$ mice, $n = 48$ cells). (D) Schematic for modified rabies virus tracing. (E and F) Images from a *Vglut2*^{ires-cre} mouse showing FLEX-TVA-mCherry expression in LH glutamatergic neurons (E) and appreciable SADΔG-GFP labeling of BNST neurons (F). (G and H) FLEX-TVA-mCherry expression in LH GABAergic neurons (G) and minimal SADΔG-GFP labeling of BNST neurons (H). Green, SADΔG-GFP; red, FLEX-TVA-mCherry; blue, Nissl stain. Scale bars, 200 μ m. (I) Significantly more BNST neurons innervate LH glutamatergic neurons compared with LH GABAergic neurons ($F_{1,20} = 38.50$, $P < 0.001$, $n = 3$ mice per group).

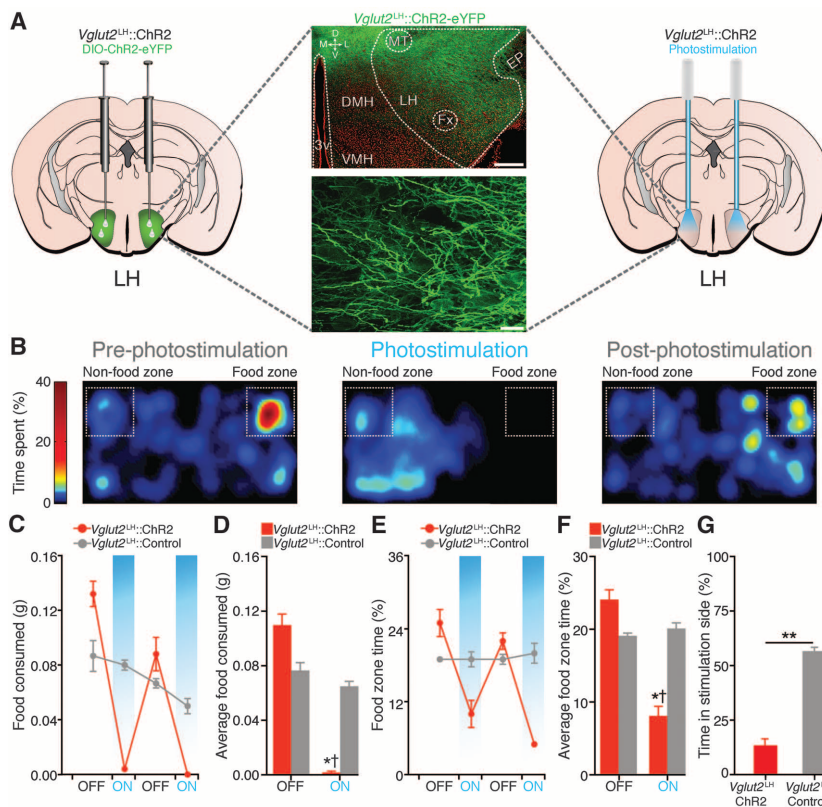


protein (RG) (AAV8-FLEX-RG) proteins that allow for rabies virus infection and subsequent transsynaptic viral propagation, respectively (23, 24), to LH-glutamatergic or -GABAergic neurons. Two weeks after adeno-associated virus (AAV) transduction, the modified rabies virus, SADΔG-GFP(EnvA), was injected into the LH, and BNST-containing slices were obtained 7 days later for confocal imaging. *Vglut2*^{LH::Rabies} tracing revealed dense populations of transsynaptically labeled BNST neurons (Fig. 3, E and F), whereas *Vgat*^{LH::Rabies} tracing resulted in minimal BNST labeling (Fig. 3, G to I, and fig. S8).

Because BNST-GABAergic projection neurons promote feeding and selectively target LH glutamatergic neurons, we considered *Vglut2*^{LH} neurons as a critical downstream circuit node for regulating food intake. Photoactivation of *Vglut2*^{LH} neurons (Fig. 4A and fig. S9) suppressed feeding in food-deprived mice (Fig. 4, B to F, and fig. S10), whereas photoinhibition of *Vglut2*^{LH} neurons induced feeding in well-fed mice (figs. S11 to S13). Photostimulation of *Vglut2*^{LH} neurons produced aversion (Fig. 4G and fig. S10), and *Vglut2*^{LH} inhibition produced a preference for palatable foods (table S1).

Until now, the precise neurocircuit elements responsible for the feeding and reinforcement phenomena observed five decades ago by electrical stimulation of the LH (1, 2, 25) have remained a mystery. Inhibitory inputs from the BNST specifically innervate and suppress LH glutamatergic neurons to promote feeding. Further unraveling of the specific patterns of gene expression and projection targets of LH glutamatergic neurons could identify novel points for therapeutic intervention within these circuits for the treatment of eating disorders and obesity.

Fig. 4. Photoactivation of *Vglut2^{LH}* neurons suppresses feeding in food-deprived mice and is aversive. (A) ChR2-eYFP expression in the LH of a *Vglut2-ires-Cre* mouse. Scale bars, 200 μ m (top), 20 μ m (bottom). (B) Spatial location heat maps in 10-min epochs before, during, and after 5-Hz photostimulation. (C and D) Photostimulation of *Vglut2^{LH}* neurons significantly decreased food intake ($F_{1,36} = 13.31$, $P < 0.001$) and food zone time (E and F) ($F_{1,36} = 13.12$, $P < 0.001$, $n = 5$ mice per group). (G) *Vglut2^{LH}::ChR2* mice spent significantly less time in the photostimulation-paired side when compared with controls ($P < 0.001$, $n = 5$ mice per group).



References and Notes

- B. G. Hoebel, P. Teitelbaum, *Science* **135**, 375–377 (1962).
- J. M. Delgado, B. K. Anand, *Am. J. Physiol.* **172**, 162–168 (1953).
- R. A. Wise, *Science* **162**, 377–379 (1968).
- B. G. Stanley, L. H. Ha, L. C. Spears, M. G. Dee II, *Brain Res.* **613**, 88–95 (1993).
- C. I. Turenius et al., *Brain Res.* **1262**, 16–24 (2009).
- J. D. Hahn, L. W. Swanson, *J. Comp. Neurol.* **520**, 1831–1890 (2012).
- J. S. de Olmos, L. Heimer, *Ann. N.Y. Acad. Sci.* **877**, 1–32 (1999).
- J. H. Jennings et al., *Nature* **496**, 224–228 (2013).
- S.-Y. Kim et al., *Nature* **496**, 219–223 (2013).
- T. Kudo et al., *J. Neurosci.* **32**, 18035–18046 (2012).
- M. Angeles-Castellanos, J. Mendoza, C. Escobar, *Neuroscience* **144**, 344–355 (2007).
- P. M. Johnson, P. J. Kenny, *Nat. Neurosci.* **13**, 635–641 (2010).
- B. Y. Chow et al., *Nature* **463**, 98–102 (2010).
- J. Mattis et al., *Nat. Methods* **9**, 159–172 (2011).
- M. M. Karmali, G. Szabo, F. Erdelyi, D. Burdakov, *J. Physiol.* **591**, 933–953 (2013).
- Z. A. Knight et al., *Cell* **151**, 1126–1137 (2012).
- G. M. Leininger et al., *Cell Metab.* **14**, 313–323 (2011).
- T. Sakurai et al., *Cell* **92**, 573–585 (1998).
- R. Sharf et al., *Biol. Psychiatry* **67**, 753–760 (2010).
- L. W. Swanson, G. Sanchez-Watts, A. G. Watts, *Neurosci. Lett.* **387**, 80–84 (2005).
- D. Atasoy, J. N. Betley, H. H. Su, S. M. Sternson, *Nature* **488**, 172–177 (2012).
- H.-R. Berthoud, H. Münzberg, *Physiol. Behav.* **104**, 29–39 (2011).
- M. Watabe-Uchida, L. Zhu, S. K. Ogawa, A. Vamanrao, N. Uchida, *Neuron* **74**, 858–873 (2012).
- I. R. Wickersham, S. Finke, K.-K. Conzelmann, E. M. Callaway, *Nat. Methods* **4**, 47–49 (2007).
- J. Olds, P. Milner, *J. Comp. Physiol. Psychol.* **47**, 419–427 (1954).

Acknowledgments: We thank the Stuber laboratory and C. Bulik for discussion. We thank K. Deisseroth and E. Boyden for viral constructs and the University of

North Carolina (UNC) Vector Core for viral packaging, B. Lowell and L. Vong for mice, and the UNC Neuroscience Center Microscopy Core (P30 NS045892). This study was supported by the Klarman Family Foundation, the Brain and Behavior Research Foundation, the Foundation of Hope, and the National Institute on Drug Abuse (DA032750) and the National Institute on Alcohol Abuse and Alcoholism (AA022234 and AA011605). A.M.S. was supported by NS007431 and DA034472.

Supplementary Materials

www.sciencemag.org/content/341/6153/1517/suppl/DC1
Materials and Methods
Figs. S1 to S13
Tables S1 and S2
Movie S1
References (26, 27)

12 June 2013; accepted 28 August 2013
10.1126/science.1241812

Cocaine Disinhibits Dopamine Neurons by Potentiation of GABA Transmission in the Ventral Tegmental Area

Christina Bocklisch,^{1*} Vincent Pascoli,¹ Jovi C. Y. Wong,^{1†} David R. C. House,¹ Cédric Yvon,¹ Mathias de Roo,¹ Kelly R. Tan,¹ Christian Lüscher^{1,2‡}

Drug-evoked synaptic plasticity in the mesolimbic system reshapes circuit function and drives drug-adaptive behavior. Much research has focused on excitatory transmission in the ventral tegmental area (VTA) and the nucleus accumbens (NAc). How drug-evoked synaptic plasticity of inhibitory transmission affects circuit adaptations remains unknown. We found that medium spiny neurons expressing dopamine (DA) receptor type 1 (D1R-MSNs) of the NAc project to the VTA, strongly preferring the GABA neurons of the VTA. Repeated in vivo exposure to cocaine evoked synaptic potentiation at this synapse, occluding homosynaptic inhibitory long-term potentiation. The activity of the VTA GABA neurons was thus reduced and DA neurons were disinhibited. Cocaine-evoked potentiation of GABA release from D1R-MSNs affected drug-adaptive behavior, which identifies these neurons as a promising target for novel addiction treatments.

Disinhibition, the removal of an inhibitory brake on neuronal firing, may affect circuit function in several parts of the brain

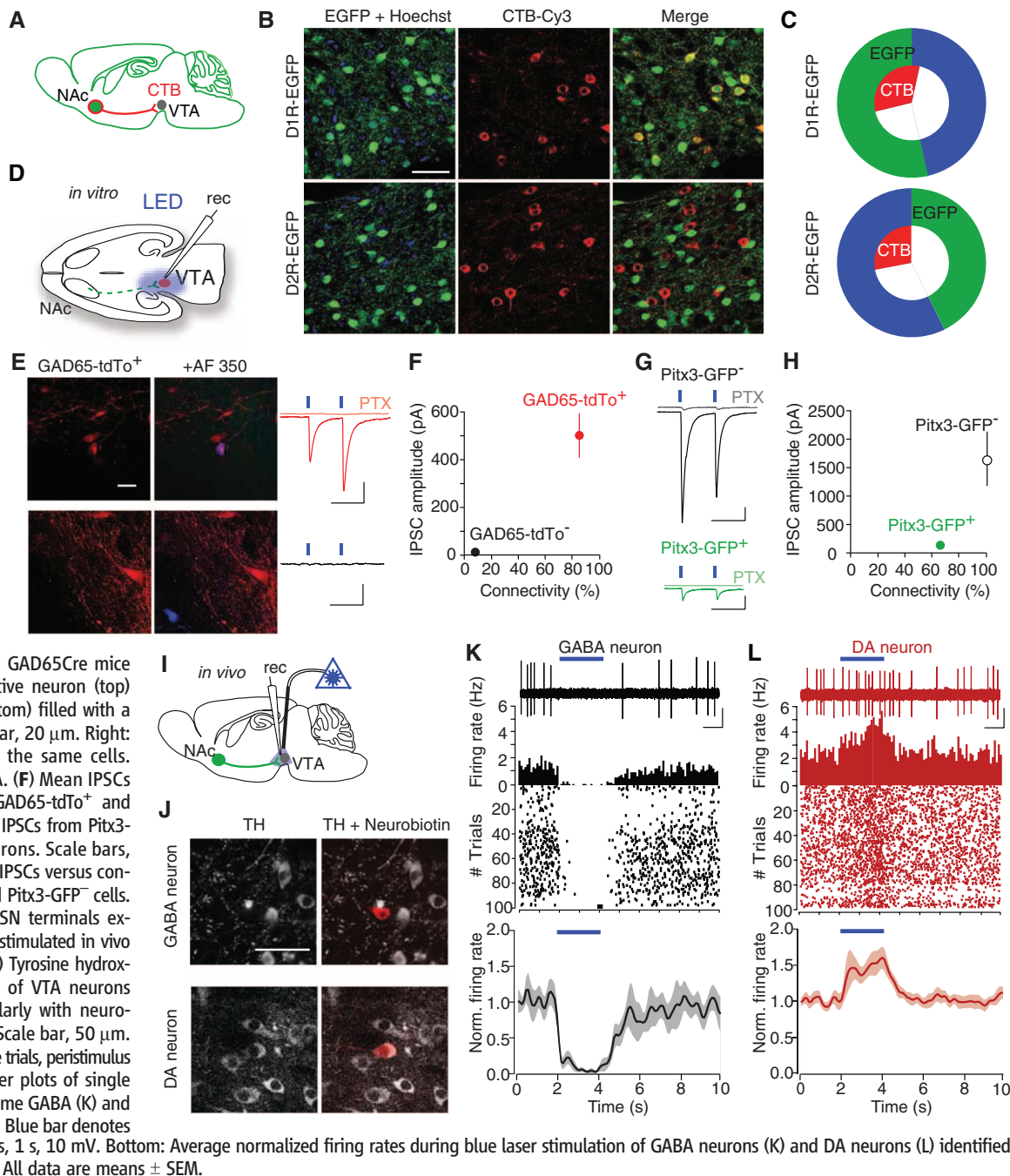
(1–3). Disinhibition of ventral tegmental area (VTA) DA neurons has been implicated in drug reinforcement when, in the acute phase, the addictive

drug shuts down VTA γ -aminobutyric acid (GABA) neurons (4–6). To understand how this monosynaptic building block integrates into the larger circuitry, we characterized the functional anatomy

of the inhibitory projections to the VTA. We focused on the major input that originates in the nucleus accumbens (NAc). Accumbal medium spiny neurons (MSNs) fall into two classes, the D1R-MSNs and D2R-MSNs (7), which may segregate with the projection target. To reveal the type of MSN projecting to the midbrain, we injected a retrograde tracer (B subunit of cholera toxin) fused to a fluorescent cyanine dye (CTB-Cy3) into the VTA of bacterial artificial chromosome (BAC) transgenic mice in which the expression of enhanced green fluorescent protein (EGFP) is driven by the promoters of D1 or D2 receptors, respectively (D1R-EGFP and D2R-EGFP mice; Fig. 1A). We then counted the CTB-Cy3–

positive cells and determined the colocalization with EGFP in neurons of the NAc of both mouse lines (Fig. 1B; Hoechst, a nuclear stain, was used to obtain the total number of cells, $n = 1721$ and $n = 1922$ in D1R-EGFP and D2R-EGFP mice, respectively; 3 animals each). EGFP-positive cells accounted for about half of all cells (D1R-mice, $52 \pm 2\%$; D2R-EGFP mice, $43 \pm 1\%$; Fig. 1C). A smaller fraction of cells, similar in both mouse lines, was positive for CTB-Cy3 (D1R-EGFP mice, $34 \pm 8\%$; D2R-EGFP mice, $33 \pm 4\%$; Fig. 1C). Colocalization with EGFP was observed only in D1R-EGFP mice (Fig. 1, B and C). Hence, only D1R-MSNs project directly to the midbrain.

Fig. 1. VTA-projecting MSNs express D1Rs and disinhibit VTA DA neurons in vivo by preferentially targeting VTA GABA neurons. (A) Experimental setup: Injection of retrograde tracer CTB-Cy3 into the VTA of D1R- or D2R-EGFP mice. (B) Confocal images of NAc slices from D1R-EGFP⁺ or D2R-EGFP⁺ mice (green) injected intra-VTA with CTB-Cy3 (red). Scale bar, 50 μ m. (C) EGFP⁺ (green) neurons and CTB-Cy3-labeled cells (red) as a proportion of total cell number (blue) in each mouse line. Overlapping segments represent colocalization. (D) In vitro slices. ChR2-Venus was expressed in the NAc of GAD65Cre-tdTo or Pitx3-GFP mice and VTA-projecting MSN terminals were stimulated with blue light. (E) Images of VTA slices from GAD65Cre mice showing a tdTomato-positive neuron (top) and negative neuron (bottom) filled with a blue dye (AF 350). Scale bar, 20 μ m. Right: Whole-cell recordings of the same cells. Scale bars, 50 ms, 100 pA. (F) Mean IPSCs versus connectivity of GAD65-tdTo⁺ and GAD65-tdTo⁻ neurons. (G) IPSCs from Pitx3-GFP⁻ and Pitx3-GFP⁺ neurons. Scale bars, 50 ms, 200 pA. (H) Mean IPSCs versus connectivity of Pitx3-GFP⁺ and Pitx3-GFP⁻ cells. (I) Experimental setup: MSN terminals expressing ChR2-Venus were stimulated in vivo by illuminating the VTA. (J) Tyrosine hydroxylase (TH, white) staining of VTA neurons labeled in vivo juxtacellularly with neurobiotin (red, right panels). Scale bar, 50 μ m. (K and L) Top: Representative trials, peristimulus time histograms, and raster plots of single unit recordings from the same GABA (K) and DA neuron (L) shown in (J). Blue bar denotes a 2-s laser pulse. Scale bars, 1 s, 10 mV. Bottom: Average normalized firing rates during blue laser stimulation of GABA neurons (K) and DA neurons (L) identified with juxtacellular labeling. All data are means $\pm$ SEM.



Because the VTA contains GABA and DA neurons (8) and because recent studies have suggested that MSNs of the NAc project to both (9, 10), we aimed to characterize the functional connectivity between MSNs and VTA neurons. We expressed ChR2 in the NAc of mice that expressed a fluorescent marker in VTA GABA neurons (GAD65-tdTomato) and prepared acute slices of the VTA (Fig. 1D). We recorded from both tdTomato⁺ and tdTomato⁻ neurons (Fig. 1E) and induced GABA release by wide-field illumination. Large inhibitory postsynaptic currents (IPSCs) were elicited in the majority (87.4%) of tdTomato⁺ neurons (497 ± 89 pA, $n = 17$), whereas only a small fraction of tdTomato⁻ neurons was responsive (8.3%, 20.3 ± 15.2 pA, $n = 24$; Fig. 1F). Both picrotoxin (PTX, 100 μ M, $n = 5$) and tetrodotoxin (TTX, 0.5 μ M, $n = 5$; Fig. S1) abolished the IPSCs in tdTomato⁺ neurons, confirming action potential-dependent transmitter release followed by GABA_A receptor activation (Fig. 1E). Clearly, accumbal MSNs exert strong inhibition onto VTA GABA neurons. To reveal a possible functionally weaker connectivity onto DA neurons, we expressed the more efficient ChR2(H134R) (11) into the NAc of Pitx3-GFP mice (a marker for DA neurons) (12). Under these circumstances, we detected small IPSCs in 67% of the Pitx3-GFP⁺ cells (127 ± 44 pA, $n = 12$) and large IPSCs in 100% of Pitx3-GFP⁺ neurons (1602 ± 473 pA, $n = 10$; Fig. 1, G and H). Thus, although a direct inhibitory projection onto VTA DA neurons exists (10), we found a much more frequent and stronger inhibitory connection onto VTA GABA cells (9). This suggested that D1R-MSNs of the NAc could drive disinhibition of VTA DA neurons (13).

We therefore performed in vivo single unit recordings of VTA neurons in response to optical stimulation of MSN terminals in the VTA in anesthetized mice (Fig. 1I). We first recorded from GABA neurons, as confirmed by juxtacellular

labeling methods and post hoc immunohistochemistry (Fig. 1J, top). All GABA neurons responded to a 2-s light pulse with decreased spiking activity ($-92.2 \pm 2\%$ of baseline activity, $n = 4$; Fig. 1K). We next recorded from DA neurons (Fig. 1J, bottom), whose activity was increased when the blue light was flashed ($146.8 \pm 10.5\%$ of baseline, $n = 7$, $P < 0.05$; Fig. 1L). Thus, despite a weak direct inhibitory connection to VTA DA neurons, D1R-MSN terminal activation leads to their disinhibition. This scenario was confirmed when we expressed the inhibitory opsin effector halorhodopsin (eNpHR3.0) selectively in VTA GABA neurons. An increase in DA neuron activity during a 2-s amber light activation was observed (Fig. S2). We conclude that NAc D1R-MSNs suppress the tonic activity of VTA GABA neurons, which disinhibits VTA DA neurons.

With addictive drugs, it is generally assumed that disinhibition is fully reversed once the drugs are eliminated. We hypothesized that the disinhibitory circuit in the VTA may undergo persistent remodeling with repetitive drug exposure. We investigated whether the synapse between D1-MSNs and VTA GABA neurons is capable of expressing activity-dependent synaptic plasticity. We elicited GABA release from axonal terminals in the VTA with blue light and recorded from VTA GABA neurons (Fig. 2A). High-frequency stimulation (HFS; Fig. 2A) of MSN terminals led to a robust potentiation of light-evoked IPSCs ($183 \pm 22\%$ of baseline, $n = 15$, $P < 0.05$; Fig. 2B). This inhibitory long-term potentiation (iLTP), which could also be induced with low Cl⁻-containing internal solution ($135 \pm 8\%$ of baseline, $n = 8$, $P < 0.01$; Fig. S3A), was associated with a decrease of the failure rate (baseline, 0.29 ± 0.07 ; after HFS, 0.2 ± 0.06 ; $n = 12$, $P < 0.01$) and changed variance ($1/CV^2$; baseline, 2.02 ± 0.54 ; after HFS, 4.23 ± 1.26 ; $n = 15$, $P < 0.01$); the reduction of the paired pulse

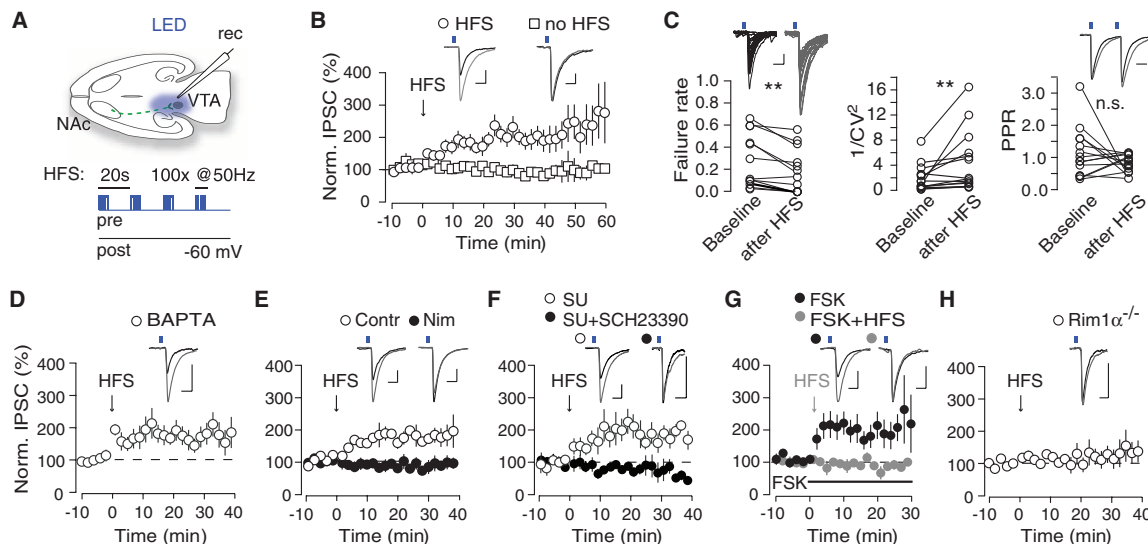
ratio (PPR) was not significant because of a high variance (baseline, 1.02 ± 0.19 ; after HFS, 0.93 ± 0.08 ; $n = 15$, $P > 0.05$; Fig. 2C). The three parameters in combination strongly suggest an increase in release probability. Infusion of a high concentration of the calcium chelator BAPTA (10 mM) into the postsynaptic neuron did not block iLTP ($160 \pm 20\%$ of baseline, $n = 14$, $P < 0.01$; Fig. 2D), whereas the calcium channel blocker nimodipine applied extracellularly (Nim, 10 μ M) or blockade of D1Rs abolished the potentiation ($91 \pm 18\%$ and $92 \pm 10\%$ of baseline, $n = 7$ and 7 , $P > 0.05$; Fig. 2, E and F).

Presynaptic forms of synaptic plasticity depend on the cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) cascade (14). We aimed to stimulate potentiation with the adenylyl cyclase (AC) activator forskolin (FSK, 10 μ M). This caused a potentiation of the transmission ($178 \pm 32\%$ of baseline, $n = 11$, $P < 0.01$; Fig. 2G, black trace), which occluded the ability of HFS to induce iLTP ($86 \pm 6\%$ of baseline, $n = 4$, $P > 0.05$; Fig. 2G, gray trace). The underlying mechanism therefore likely involves this cascade in the terminals of NAc MSNs initiated by D1Rs. We confirmed that FSK-induced potentiation is solely mediated by presynaptic PKA by additionally loading the postsynaptic cell with the membrane-impermeable PKA inhibitor PKI 6-22 (20 μ M) while bath-applying FSK (Fig. S3B). Taken together, induction as well as expression of iLTP is likely to be presynaptic, akin to well-described forms of potentiation at hippocampus mossy fiber excitatory synapses (15). Because this “sister” LTP requires the scaffolding protein Rim1 α (16), we tested its involvement in iLTP. HFS in the Rim1 α ^{-/-} mice left basal IPSC amplitudes unaffected ($97 \pm 14\%$ of baseline, $n = 7$, $P > 0.05$; Fig. 2H). Hence, iLTP in the VTA also depends on presynaptic Rim1 α .

We next wanted to test whether inhibitory transmission between D1R-MSNs and VTA

Fig. 2. HFS induces cAMP-PKA-dependent iLTP.

(A) Setup: Whole-cell recordings of light-evoked IPSCs from VTA neurons and induction protocol. (B) HFS of MSN terminals elicits iLTP. (C) Group data for failure rate, coefficient of variation ($1/CV^2$), and paired pulse ratio (PPR) at baseline and after HFS. (D and E) Intracellular BAPTA blocked iLTP (D) in contrast to bath-applied nimodipine (Nim) (E). (F) SCH23390 and sulpiride (SU) blocked iLTP. (G) FSK potentiated IPSC amplitudes and occluded iLTP. (H) No iLTP in Rim1 α ^{-/-} mice. All insets: Example traces of IPSCs at baseline (black) and after HFS (gray). Scale bars, 100 pA, 10 ms, except for (F), 40 pA, 10 ms. Data are means $\pm$ SEM.



GABA neurons was affected by repetitive drug exposure. Cocaine is a highly addictive drug that evokes synaptic plasticity at several excitatory synapses of the mesolimbic system (17). Its re-inforcing effects are mediated by increasing DA concentration in the NAc instead of disinhibiting DA neurons [cocaine actually briefly inhibits DA neuron firing in the acute phase (18)]. To test for cocaine-induced synaptic plasticity at this disinhibitory circuit, we repeatedly treated mice with cocaine [15 mg/kg intraperitoneally (i.p.) for 5 days; Fig. 3A], prepared midbrain slices 24 hours after the last injection, and examined whether this treatment interfered with the ability to elicit iLTP. Drug exposure disrupted HFS-induced iLTP (saline, $157 \pm 29\%$ of baseline, $n = 9$; cocaine, $86 \pm 7\%$ of baseline, $n = 9$; $P < 0.05$; Fig. 3B) and was associated with a decrease of the PPR (saline, 1.28 ± 0.09 , $n = 27$; cocaine, 0.82 ± 0.09 , $n = 19$; $P < 0.01$; Fig. 3C), indicating an occlusion scenario. Bath application of FSK readily potentiated light-evoked IPSCs in slices from saline-treated animals, whereas it failed to potentiate the IPSCs in the cocaine group (saline, $249 \pm 51\%$ of baseline, $n = 9$; cocaine, $101 \pm 15\%$ of baseline, $n = 8$; $P < 0.05$; Fig. 3D), confirming the involvement of the cAMP-PKA cascade in the induction of cocaine-evoked synaptic plasticity (19).

To test whether induction of cocaine-evoked inhibitory plasticity was dependent on the increase of extracellular DA concentration, we exposed mice carrying a mutated cocaine-insensitive DAT (DAT_{KI}) (20) repeatedly to cocaine. In DAT_{KI} mice, cocaine treatment did not disrupt the ex vivo iLTP ($223 \pm 38\%$ of baseline, $n = 12$, $P < 0.05$), whereas potentiation was not fully induced in heterozygous littermates ($126 \pm 23\%$ of baseline, $n = 7$, $P > 0.05$; Fig. 3E). This indicates that without DAT inhibition, cocaine does not disrupt iLTP, thus confirming that in vivo cocaine-evoked synaptic plasticity is induced by a dopamine-dependent mechanism.

The decreased PPR values suggested an enhanced GABA release probability at D1R-MSN terminals after cocaine treatment. We thus recorded spontaneous inhibitory synaptic currents (sIPSCs) after repeated cocaine treatment (Fig. 3, F to H). Cocaine increased the sIPSC frequency in GABA neurons (saline, 7.1 ± 1.1 Hz, $n = 11$; cocaine, 16.2 ± 4.1 Hz, $n = 12$, $P < 0.05$, with a trailing enhancement of the amplitudes: saline, 56 ± 5.1 pA, $n = 11$; cocaine, 72.6 ± 11 pA, $n = 12$; $P > 0.05$; Fig. 3, F and G) but not in DA neurons, where the frequency of sIPSC was in fact lower in slices from cocaine-treated mice (frequencies: saline, 14.7 ± 2.5 Hz, $n = 11$; cocaine, 7.4 ± 1.4 Hz, $n = 10$; $P < 0.05$; amplitudes: saline,

47.1 ± 3.3 pA, $n = 11$; cocaine, 40.5 ± 2.1 pA, $n = 10$; Fig. 3, F and H), in line with a depressed inhibition reported previously (21, 22). Our data suggest a long-lasting disinhibition of VTA DA neurons, most likely through the potentiation of inhibitory synaptic transmission onto GABA neurons through a presynaptic mechanism. If this is the case, then the basal firing activity of VTA DA neurons should be increased after repeated cocaine treatment. Indeed, DA neurons showed increased spiking and burst firing activity after repeated cocaine treatment relative to saline-injected mice [saline, 3.4 ± 0.6 Hz and $24.7 \pm 5.7\%$ spikes fired in bursts (SIB), $n = 23$; cocaine, 9.0 ± 1.7 Hz and $53.1 \pm 8.9\%$ SIB, $n = 16$; $P < 0.01$ and $P < 0.005$; Fig. 3I and fig. S4], whereas GABA neurons fired at lower rates after cocaine (saline, 9.9 ± 2.1 Hz, $n = 20$; cocaine, 5.1 ± 1 Hz, $n = 15$; $P < 0.05$; Fig. 3I). Elevated firing activity of DA neurons is unlikely to result from increased direct excitation onto these cells, because one single injection of cocaine, which increases glutamatergic tone onto DA neurons (23), failed to increase firing rates of DA neurons 24 hours after the injection (fig. S5). Taken together, these findings imply that cocaine selectively potentiates GABA release from NAc D1R-MSN terminals in a DA-dependent fashion such that VTA DA neurons are tonically disinhibited in the long run.

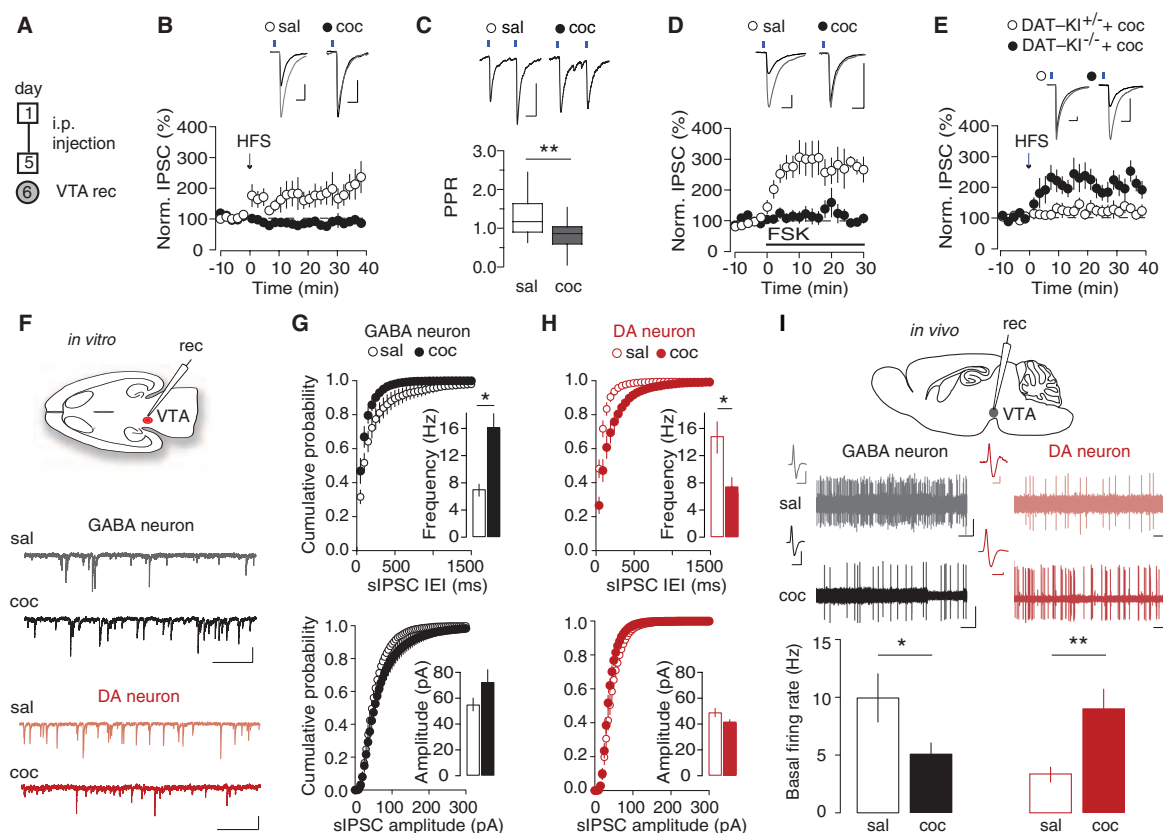


Fig. 3. Potentiation of inhibitory transmission by cocaine. (A) Treatment protocol. (B) Cocaine treatment abolishes iLTP. Scale bars, 100 pA, 10 ms. (C) PPR example traces and group data. Scale bars, 20 ms, 50 pA. (D) Occlusion of FSK potentiation by cocaine treatment. Scale bars, 100 pA, 10 ms. (E) Failure to abolish iLTP in DAT_{KI} mice. Scale bars, 100 pA, 10 ms. (F) Setup and example

traces of sIPSCs. Scale bars, 50 pA, 0.5 s. (G and H) Cumulative probability distributions of sIPSC in GABA neurons (G) and DA neurons (H). Insets: bar graphs with means $\pm$ SEM. (I) Setup and example traces from in vivo recordings of VTA GABA and DA neurons (scale bars, 2 s, 10 mV; inset scale bars, GABA, 2 ms, 10 mV; DA, 2 ms, 2 mV). Bottom: Group data for firing rates.

How may drug-evoked inhibitory plasticity relate to behavioral actions of cocaine caused by the activation of D1R-MSNs (24)? We assessed locomotor sensitization (Fig. 4A), a model for incentive saliency of cocaine that develops over five daily injections, just as cocaine-evoked inhibitory plasticity (25). We first verified the consequences of the iLTP induction protocol in vivo on the activity of VTA neurons (Fig. 4A) and observed inversed firing frequencies in GABA and DA neurons (GABA HFS versus no HFS, 2.5 ± 1 Hz and 13 ± 2 Hz, $P < 0.0001$; DA HFS versus no HFS, 9.5 ± 6 Hz and 3 ± 1 Hz, $P < 0.01$; 35 ± 12 SIB versus $16 \pm 4\%$ SIB, $P < 0.05$; $n = 10$ to 17; Fig. 4B and fig. S4), comparable to the cocaine treatment. On subsequent days, locomotor sensitization to cocaine was enhanced (control AAV, 1389 ± 184 turns/hour, $n = 15$; ChR2-AAV, 3104 ± 555 turns/hour, $n = 11$; $P < 0.01$; Fig. 4C). We next tested the effect of an in vivo HFS on conditioned place preference (CPP), a paradigm to measure the memory effect of drug reward (26). HFS was applied outside the conditioning chamber 1 day before the drug administration (10 mg/kg), which prevented CPP (control AAV, 105 ± 29 min, $n = 9$; ChR2-AAV, 17 ± 27 , $n = 11$; $P < 0.05$; Fig. 4D). These data support a model whereby the VTA serves as a gate for downstream circuit adaptations that un-

derlie sensitization and occludes CPP where cue association requires burst activity of DA neurons.

The cellular correlate of the disinhibition of VTA DA neurons is a form of homosynaptic potentiation of GABA transmission onto VTA GABA neurons. This cocaine-evoked inhibitory plasticity is DA-dependent and is expressed presynaptically. As a consequence, VTA DA neurons fire at higher frequencies, which facilitates the induction of locomotor sensitization and occludes CPP. This adaptation of the inhibitory limb of the mesolimbic circuitry occurs in parallel with a strengthening of excitatory afferents onto DA neurons in the VTA (23, 27, 28). Then, after several days of withdrawal, excitatory transmission in the NAc also adapts (26). Thus, the drug-evoked synaptic plasticity in back-projecting D1R-MSNs, described in the present study, emerges as a crucial step in circuit remodeling (29). Note that D1R-MSNs undergo presynaptic and postsynaptic changes (30), resulting in an overall strengthening of their inhibitory effects and enhanced locomotor sensitization, whereas inhibition of D1R-MSNs attenuates this behavior (31).

Drug-evoked inhibitory plasticity may also prevent further associative learning and may lead to behavioral adaptations such as compulsive cocaine seeking or incubation of craving (32, 33). Clearly, a picture is emerging of mesolimbic cir-

cuit remodeling that affects excitatory as well as inhibitory transmission, with the effect of enhancing D1R-MSN function. Controlling the activity of the D1R-MSNs, by pharmacological means or neuromodulation, may therefore emerge as an appealing target for novel therapeutic interventions in addiction.

References and Notes

1. J. J. Letzkus *et al.*, *Nature* **480**, 331–335 (2011).
2. F. Gambino, A. Holtmaat, *Neuron* **75**, 490–502 (2012).
3. R. C. Froemke, M. M. Merzenich, C. E. Schreiner, *Nature* **450**, 425–429 (2007).
4. S. W. Johnson, R. A. North, *J. Neurosci.* **12**, 483–488 (1992).
5. K. R. Tan *et al.*, *Nature* **463**, 769–774 (2010).
6. H. G. Cruz *et al.*, *Nat. Neurosci.* **7**, 153–159 (2004).
7. J. Bertran-Gonzalez *et al.*, *J. Neurosci.* **28**, 5671–5685 (2008).
8. S. R. Sesack, A. A. Grace, *Neuropsychopharmacology* **35**, 27–47 (2010).
9. Y. Xia *et al.*, *J. Neurosci.* **31**, 7811–7816 (2011).
10. M. Watabe-Uchida, L. Zhu, S. K. Ogawa, A. Vamanrao, N. Uchida, *Neuron* **74**, 858–873 (2012).
11. G. Nagel *et al.*, *Curr. Biol.* **15**, 2279–2284 (2005).
12. M. P. Smidt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13305–13310 (1997).
13. K. R. Tan *et al.*, *Neuron* **73**, 1173–1183 (2012).
14. M. G. Weisskopf, P. E. Castillo, R. A. Zalutsky, R. A. Nicoll, *Science* **265**, 1878–1882 (1994).
15. P. E. Castillo, *Cold Spring Harb. Perspect. Biol.* **4**, a005728 (2012).
16. P. E. Castillo, S. Schoch, F. Schmitz, T. C. Südhof, R. C. Malenka, *Nature* **415**, 327–330 (2002).
17. C. Lüscher, R. C. Malenka, *Neuron* **69**, 650–663 (2011).
18. M. S. Brodie, T. V. Dunwiddie, *Naunyn-Schmiedeberg Arch. Pharmacol.* **342**, 660–665 (1990).
19. D. W. Self *et al.*, *J. Neurosci.* **18**, 1848–1859 (1998).
20. R. Chen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9333–9338 (2006).
21. B. Pan, C. J. Hillard, Q.-S. Liu, *J. Neurosci.* **28**, 1385–1397 (2008).
22. Q.-S. Liu, L. Pu, M.-M. Poo, *Nature* **437**, 1027–1031 (2005).
23. M. A. Ungless, J. L. Whistler, R. C. Malenka, A. Bonci, *Nature* **411**, 583–587 (2001).
24. M. K. Lobo *et al.*, *Science* **330**, 385–390 (2010).
25. T. E. Robinson, K. C. Berridge, *Brain Res. Brain Res. Rev.* **18**, 247–291 (1993).
26. H. L. Fields, G. O. Hjelmstad, E. B. Margolis, S. M. Nicola, *Annu. Rev. Neurosci.* **30**, 289–316 (2007).
27. C. Bellone, C. Lüscher, *Nat. Neurosci.* **9**, 636–641 (2006).
28. M. T. C. Brown *et al.*, *PLoS ONE* **5**, e15870 (2010).
29. D. Belin, B. J. Everitt, *Neuron* **57**, 432–441 (2008).
30. V. Pascoli, M. Turiault, C. Lüscher, *Nature* **481**, 71–75 (2012).
31. R. Chandra *et al.*, *Front. Mol. Neurosci.* **6**, 13 (2013).
32. J. W. Grimm, B. T. Hope, R. A. Wise, Y. Shaham, *Nature* **412**, 141–142 (2001).
33. M. Mamellet *et al.*, *Nat. Neurosci.* **12**, 1036–1041 (2009).

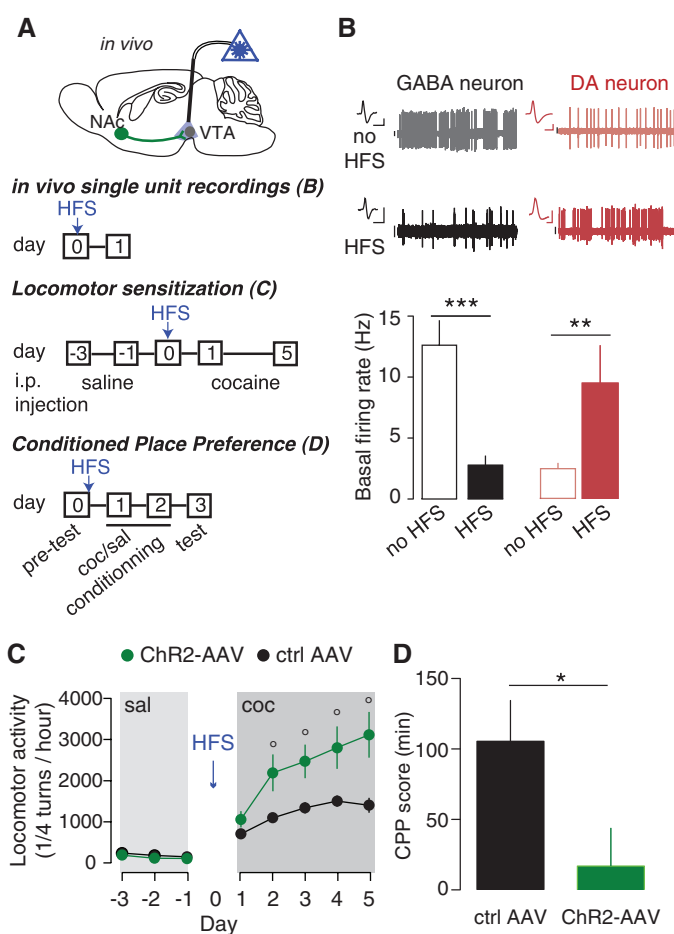
Acknowledgments: We thank M. Serafini and the members of the Lüscher laboratory for suggestions on the manuscript, M. Brown for support during the in vivo electrophysiology experiments, G. Miesenböck for GADCre mice, and S. Schoch for Rim1a^{-/-} mice. Supported by the Swiss National Science Foundation, the European Research Council, and “Synapsy,” a National Competence Center in Research (C.L.) and by a Swiss Confederation scholarship (J.C.Y.W.).

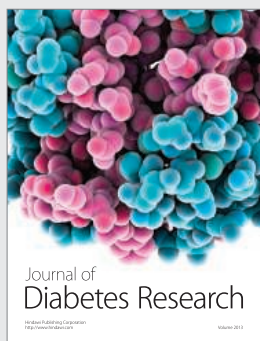
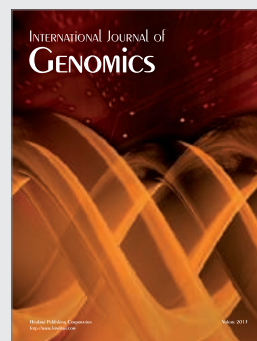
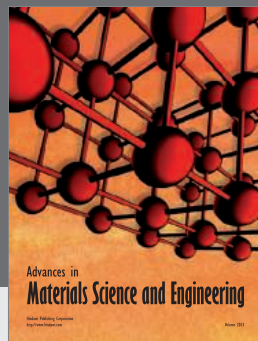
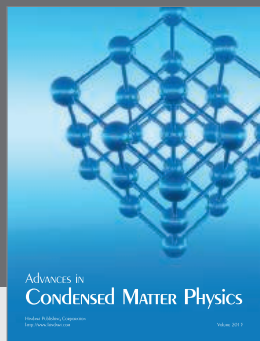
Supplementary Materials

www.sciencemag.org/content/341/6153/1521/suppl/DC1
Materials and Methods
Figs. S1 to S5

26 February 2013; accepted 5 September 2013
10.1126/science.1237059

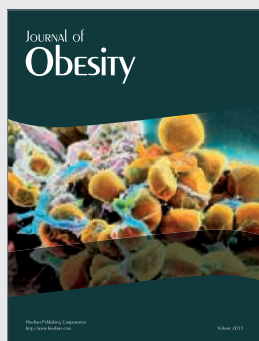
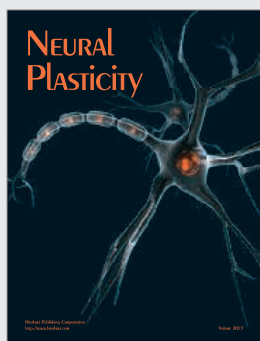
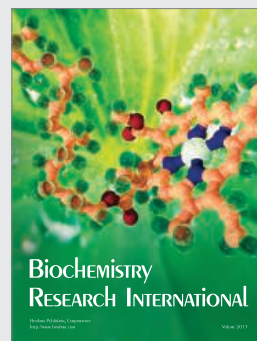
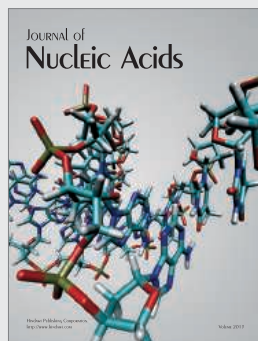
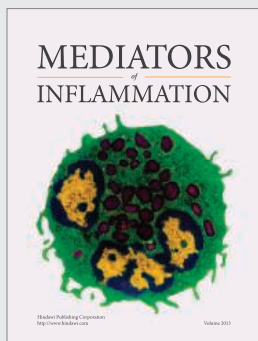
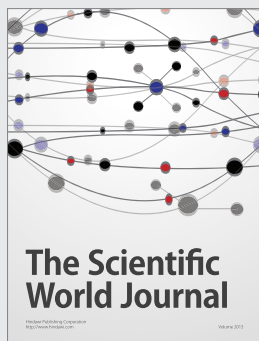
Fig. 4. In vivo HFS enhances cocaine-induced locomotor sensitization and occludes conditioned place preference. (A) Experimental setup. (B) Example traces of in vivo recordings of VTA GABA and DA neurons (scale bars, 5 mV; inset scale bars, 2 ms, 10 mV). Bottom: Group data for firing rates. (C) Locomotor activity immediately after saline (days –3 to –1) or cocaine (days 1 to 5) injections (i.p.). (D) Group data for CPP score. Data are means $\pm$ SEM.





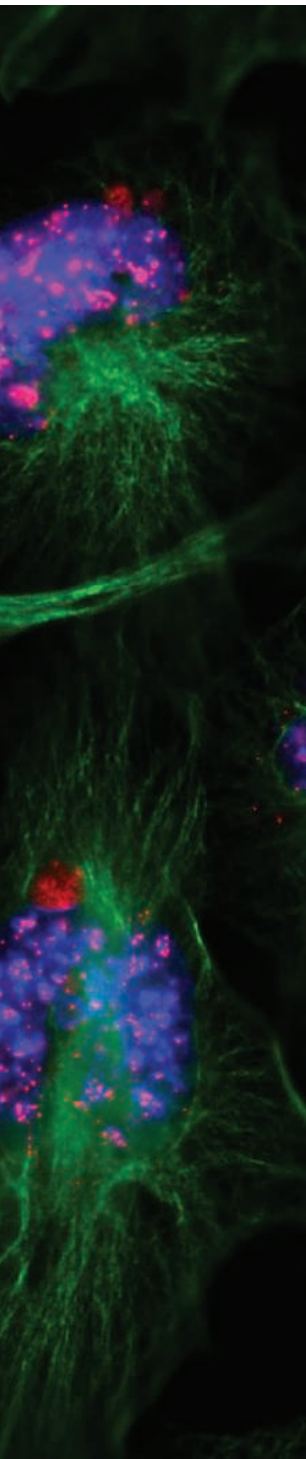
Hindawi

Submit your manuscripts at
<http://www.hindawi.com>



SCIENTIFIC CONFERENCES 2013-2014:

Presenting the most significant research on cancer etiology, prevention, diagnosis, and treatment



AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics

Co-Chairpersons: Jeffrey A. Engelman, Lee J. Helman, and Sabine Tejpar
October 19-23, 2013 • Boston, MA

Twelfth Annual International Conference on Frontiers in Cancer Prevention Research

Chairperson: Paul J. Limburg
October 27-30, 2013 • National Harbor, MD

Pediatric Cancer at the Crossroads: Translating Discovery into Improved Outcomes

Co-Chairpersons: John M. Maris, Stella M. Davies, James R. Downing, Lee J. Helman, and Michael B. Kastan
November 3-6, 2013 • San Diego, CA

The Translational Impact of Model Organisms in Cancer

Co-Chairpersons: Cory Abate-Shen, A. Thomas Look, and Terry A. Van Dyke
November 5-8, 2013 • San Diego, CA

Ninth Annual Personalized Medicine Conference

Chairperson: Raju Kucherlapati
November 6-7, 2013 • Boston, MA
Advance registration deadline: **Friday, October 11**

Sixth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

Co-Chairpersons: John D. Carpten, Christopher I. Li, and Olufunmilayo I. Olopade
December 6-9, 2013 • Atlanta, GA
Advance registration deadline: **Thursday, October 24**

CTRC-AACR San Antonio Breast Cancer Symposium

Co-Directors: Carlos L. Arteaga, C. Kent Osborne, and Peter M. Ravdin
December 10-14, 2013 • San Antonio, TX
Early registration deadline: **Thursday, October 31**

AACR-IASLC Joint Conference on Molecular Origins of Lung Cancer

Co-Chairpersons: Roy Herbst, Elisabeth Brambilla, Pasi Jänne, and William Pao
January 6-9, 2014 • San Diego, CA
Abstract submission and award application deadline: **Monday, October 14**
Advance registration deadline: **Tuesday, November 26**

AACR-Prostate Cancer Foundation Conference on Advances in Prostate Cancer Research

Co-Chairpersons: Arul M. Chinnaiyan, William G. Nelson, June M. Chan, and Jonathan W. Simons
January 18-21, 2014 • San Diego, CA

Cancer Susceptibility and Cancer Susceptibility Syndromes

Co-Chairpersons: Alan D. D'Andrea, Phillip A. Dennis, and Pier Paolo Pandolfi
January 29-February 1, 2014 • San Diego, CA
Abstract submission deadline: **Wednesday, November 13**
Advance registration deadline: **Monday, December 9**

RAS Oncogenes: From Biology to Therapy

Co-Chairpersons: Frank McCormick, Dafna Bar-Sagi, and Channing J. Der
February 24-27, 2014 • Lake Buena Vista, FL
Abstract submission and award application deadline: **Friday, December 6**
Advance registration deadline: **Monday, January 13**

Cellular Heterogeneity in the Tumor Microenvironment

Co-Chairpersons: Mary Helen Barcellos-Hoff, Michele De Palma, and M. Celeste Simon
February 26-March 1, 2014 • San Diego, CA
Abstract submission and award application deadline: **Monday, December 16**
Advance registration deadline: **Monday, January 13**

AACR Annual Meeting 2014

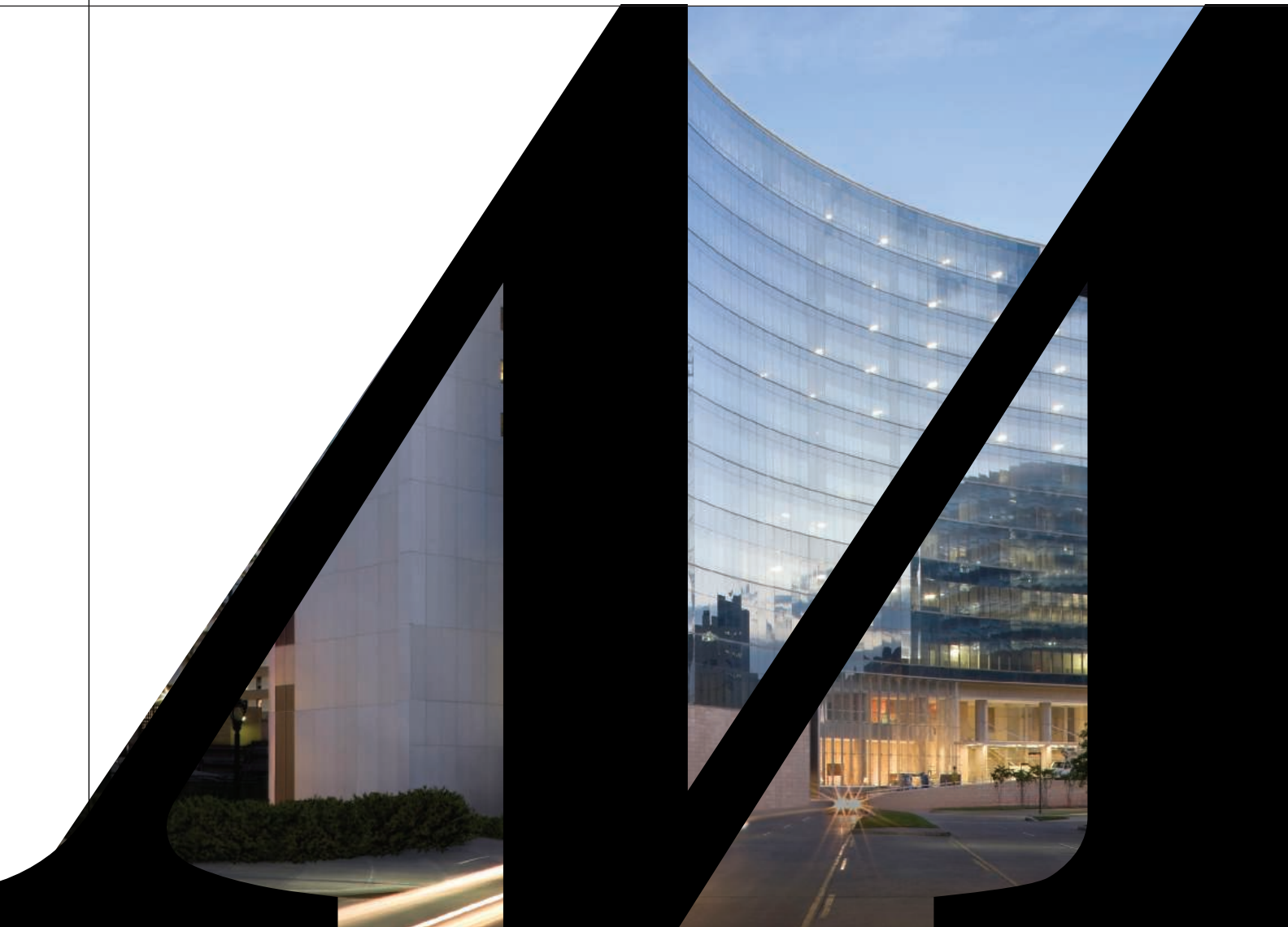
Chairperson: Scott W. Lowe
April 5-9, 2014 • San Diego, CA

NEW NAME. NEW ADVANCES. THE METHODIST HOSPITAL IS NOW HOUSTON METHODIST HOSPITAL.

We've changed our name. The Methodist Hospital is now Houston Methodist Hospital. We're always moving forward, and we believe that our greatest advances are those that are just around the corner. So we continue to look toward the future, bringing the finest researchers and clinicians from around the world to Houston, to join us in the pursuit of better care and better cures. That's the difference between practicing medicine and leading it.

houstonmethodist.org

HOUSTON
MethodistSM
LEADING MEDICINE





Always had a global view?

MAKE GREAT THINGS HAPPEN

Opportunities for research scientists and professionals: Looking beyond borders has been your dream since childhood days? We offer extraordinary global career perspectives for scientists in our Research & Development hubs in Greater Boston (USA), Greater Frankfurt (Germany), Beijing (China) or Tokio (Japan). Contribute to challenging research projects aimed at fighting disease worldwide. Join us and take part in shaping our diversified bio pharma business. Ready for an exploration journey?

EMD Serono is the biopharmaceutical subsidiary of Merck KGaA, Darmstadt, Germany in the U.S.

With more than 300 years of progress and about 38,000 employees in over 60 countries, we are leading in pharma, chemicals and life sciences. With passion, dedication and innovative ideas, we pursue one global goal: to improve people's quality of life. Like to join in? Welcome to the team!

come2emd.com



"What I do with my Octet HTX time? Ride."

Epitope binning studies in days, not weeks.

Are your antibody epitope binning experiments just getting bigger and bigger? The new Octet HTX system lets you get them done in days, not weeks. Start a 32 x 32 cross-competition matrix in the morning, then analyze your data with a few mouse clicks before you leave the lab for the day. Can your ELISA or SPR system do that?

Lauren gets out of the lab more often now to ride. What will you do with your extra time?



fortéBIO
A Division of **Pall Life Sciences**

PALL Life Sciences

fortebio.com | 888-OCTET-75

Fast. Accurate. EASY.



Join the Conversation!

Twitter is a great way to connect with AAAS members and staff about the issues that matter to you most. Be a part of the discussion while staying up-to-date on the latest news and information about your personal member benefits.

**Follow us @AAASmember
and join the conversation
with #AAAS**



MemberCentral.aaas.org

Where Will Your Research Be When Film Goes Away?



Now available
\$4,995*



**Everything you love about film,
without the hassles!**

Introducing Digital Film for Chemiluminescent Western Blots
with the C-Digit® Blot Scanner from LI-COR.

*MSRP. Price varies. © 2013 LI-COR, Inc.

Visit www.mycdigit.com for more information

LI-COR



AAAS is here –

bringing educational infrastructure
to the developing world.

AAAS is helping the Rwandan government rebuild its educational infrastructure as a way to help drive economic growth and development. By providing materials such as the Project 2061 *Atlas of Science Literacy*, lesson plans from Science NetLinks, and access to *Science* digital libraries, AAAS is helping the people of Rwanda work toward a future built around science and technology. As a AAAS member your dues support these efforts. If you're not yet a AAAS member, join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/rwanda



AAAS Travels

Peru & Easter Island
January 23–February 6, 2014

With Dr. Douglas Sharon



Explore the Cultural Heritage of Peru & Easter Island!

Explore the finest archaeological and historic sites in Peru & Easter Island including the classic Inca sites in the Andes, and the ancient coastal cultures north of Lima. Explore the Moche civilization near Trujillo and visit Chan-Chan. On Easter Island another rich cultural landscape awaits you quite different from Peru including majestic moais. \$4,895 + air

**For a detailed brochure,
please call (800) 252-4910**

All prices are per person twin share + air



BETCHART EXPEDITIONS Inc.
17050 Montebello Rd, Cupertino, CA 95014
Email: AAASInfo@betchartexpeditions.com
www.betchartexpeditions.com



The New Legend

Eppendorf Reference® 2

»Reference« stands for extraordinary precision and accuracy, a long service life, and an ergonomic design. The new Reference 2 boasts these proven Premium characteristics and this operating philosophy with its innovative state-of-the-art technology; making it a reliable partner for you and your demanding work.

- > Single-button operation enables ergonomic handling with reduced operating effort
- > High precision and accuracy providing reliable pipetting results
- > Quick and secure volume setting, incl. volume lock
- > RFID chip contains all relevant data regarding the pipette



www.eppendorf.com/reference

Eppendorf®, the Eppendorf logo, Eppendorf PhysioCare Concept® and Eppendorf Reference® are registered Trademarks of Eppendorf AG, Germany. All right reserved, including graphics and images. Copyright © 2013 by Eppendorf AG.

Redefining the Link Between Basic and Applied Science



The Graduate School of Biomedical Sciences

at the Icahn School of Medicine at Mount Sinai in New York City offers PhD and MD/PhD students the opportunity to earn their degrees in Biomedical Sciences or Neurosciences in one of the most innovative environments in the country.



Icahn
School of
Medicine at
Mount
Sinai



- **Ranked #2 by U.S. News & World Report** among the 30 freestanding medical schools with an associated graduate school
- **Ranked #5 in the nation** for National Institutes of Health funding per investigator
- **The Graduate School's faculty** are part of more than 200 diverse research laboratories

The Graduate School of Biomedical Sciences

Icahn School of Medicine at Mount Sinai

FIND OUT MORE:

www.ichn.mssm.edu/gradschool

EMAIL: grads@mssm.edu

SCIENCE & DIPLOMACY

SCIENCE & DIPLOMACY

provides an open access forum for rigorous thought, analysis, and insight to serve stakeholders who develop, implement, or teach all aspects of science and diplomacy. Learn more about the latest ideas in science diplomacy and receive regular updates by following @SciDip on Twitter and registering for free at www.sciencediplomacy.org/user/register.



WWW.SCIENCEDIPLOMACY.ORG

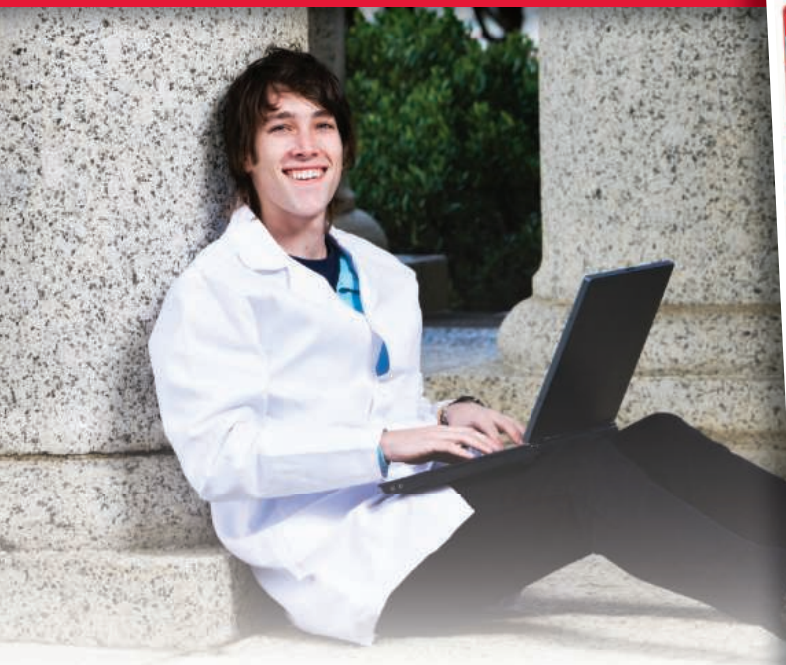
Science & Diplomacy is published by the Center for Science Diplomacy of the American Association for the Advancement of Science (AAAS), the world's largest general scientific society.

SCIENCE &
DIPLOMACY



For your career in science, there's only one **Science**

A career plan customized
for you, by you.



myIDP.sciencecareers.org



Recommended by leading professional societies and endorsed by the National Institutes of Health, an individual development plan will help you prepare for a successful and satisfying scientific career.



In collaboration with FASEB, UCSF, and the Medical College of Wisconsin and with support from the Burroughs Wellcome Fund, AAAS and *Science* Careers present the first and only online app that helps scientists prepare their very own individual development plan.

Visit the website and
start planning today!
myIDP.sciencecareers.org

In partnership with:



HEAT FLOW CALORIMETER

OptiMax HFCal is a newly designed heat flow calorimeter that offers highly intuitive operation so users can run experiments faster for significant resource savings. A chemical reaction's heat-related information is key to developing a safe, robust process—and the earlier this information can be evaluated, the faster the process can be transferred into large-scale production. OptiMax HFCal enables easy, rapid characterization of these process-safety parameters, including heat transfer, specific heat of reaction mass, isothermal and nonisothermal heat flow, enthalpy, and thermal conversion rates at all reaction stages. The electrical heating and Peltier cooling systems ensure precise temperature control from -40°C to 180°C without cryostat and ice-bath maintenance for added time savings and a smaller bench footprint. Easy data gathering is also critical for establishing reliable, repeatable calorimetric measurement. With OptiMax HFCal, all experiment data is recorded automatically, making processes traceable and reproducible.

Mettler Toledo

For info: 800-638-8537 | www.mt.com/OptiMaxHFCal



SOLID PHASE EXTRACTION

Thermo Fisher Scientific is pleased to announce the additions of WAX and WCX phases for bioanalysis to its revolutionary SOLA solid phase extraction (SPE) product line. The new phases are available in cartridge and plate formats. Thermo Scientific SOLA products are the first fritless SPE cartridges and plates, offering: unsurpassed reproducibility at low elution volumes, low failure rates, enhanced sensitivity, and clean sample extracts. The novel design eliminates voiding, channeling, and packing inconsistencies that can cause analytical failures or poor reproducibility.

Thermo Fisher Scientific

For info: 408-965-6408 | www.thermoscientific.com/sola-SPE

PURIFICATION COLUMNS

The new cOmplete His-Tag Purification Column for researchers performing histidine-tagged protein purification from lysates. The new column uses Roche's proprietary nickel-chelate chemistry and is compatible with commonly used reducing agents (DTT), metalloproteinase-inhibiting reagents such as EDTA, and different buffer and salt environments. This allows researchers to choose optimal buffer conditions for the target protein in a convenient prepacked format. Following the purification step, the new column does not require buffer exchange or resin recharging therefore avoiding a costly and time consuming process. In addition, the resin's minimized nickel ion leakage not only reduces toxic nickel waste, but also stabilizes the target protein by preventing nickel ions from catalyzing protein oxidation. Thanks to the additional format, researchers can choose their preferred method of purification. The cOmplete His-Tag Purification Resin can be used for batch purification as well as on automated systems based on fast protein liquid chromatography. Prepacked cOmplete His-Tag Purification Columns are available in two sizes (1 mL and 5 mL) and are compatible with standard instruments for protein purification.

Roche

For info: 800-262-1640 | www.complete.roche.com

STABILIZED PEPSIN

The new CytoZyme Stabilized Pepsin is an advanced formulation of pepsin for pretreating tissue samples in nucleic acid probe-based cytogenetic assays including fluorescence in situ hybridization (FISH). CytoZyme is a ready-to-use solution of purified pepsin that can be stored in the refrigerator without loss of activity. It replaces dry powder forms of pepsin that vary in activity, which can negatively affect test results. Variability in FISH/ISH test results is frequently traceable to the protease used to pretreat patient samples prior to hybridization with nucleic acid probes. CytoZyme provides reliable activity day-to-day and lot-to-lot thereby eliminating variation in protease treatment as a common source of test variability in cytogenetic labs.

SciGene

For info: 408-733-7337 | www.scigene.com

CHROMATOGRAPHY COLUMNS

The Superdex 200 Increase size-exclusion chromatography columns are designed for both preparative and analytical purposes for a broad range of biomolecules, enabling higher purity and improved protein analysis. The columns deliver up to double the resolution or retained resolution in half the time, depending on the conditions selected and the molecule. Optimized for monoclonal antibody separation, the Superdex 200 Increase prepacked columns can be used for small-scale (μg – mg) high-resolution gel filtration and for characterizing a variety of different proteins sized between 10,000 Da and 600,000 Da. The column's high-flow agarose base matrix is pH stable, easy to clean, and has low nonspecific interaction. Superdex 200 Increase is available in three sizes (10/300 GL, 5/150 GL, and 3.2/300) and can be used with ÄKTA chromatography systems for a variety of applications including protein purification, aggregate analysis, studies of complex formation, and screening of samples.

GE Healthcare Life Sciences

For info: 800-526-3593 | www.gelifesciences.com/newsec

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.

Support the sciences. **Get rewarded.**

Show your AAAS pride and reward yourself with the new AAAS Platinum Advantage Rewards Card from NASA Federal Credit Union.

Apply now and get
10,000 bonus points!

Go to nasafcu.com/AAASpromo



Get **10,000 bonus points** if you sign up for a card and spend \$3,000 within 90 days of account opening.

Learn more at
nasafcu.com/AAASpromo.

Subject to credit approval.
Membership in AAAS and NASA FCU is required.
NASA FCU is federally insured by NCUA.



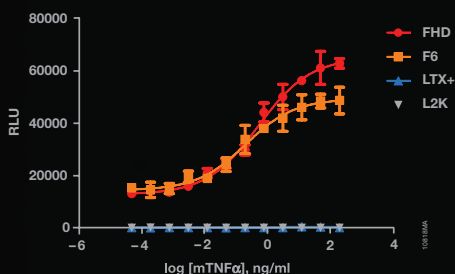
FuGENE[®] HD

Illuminate Real Biology

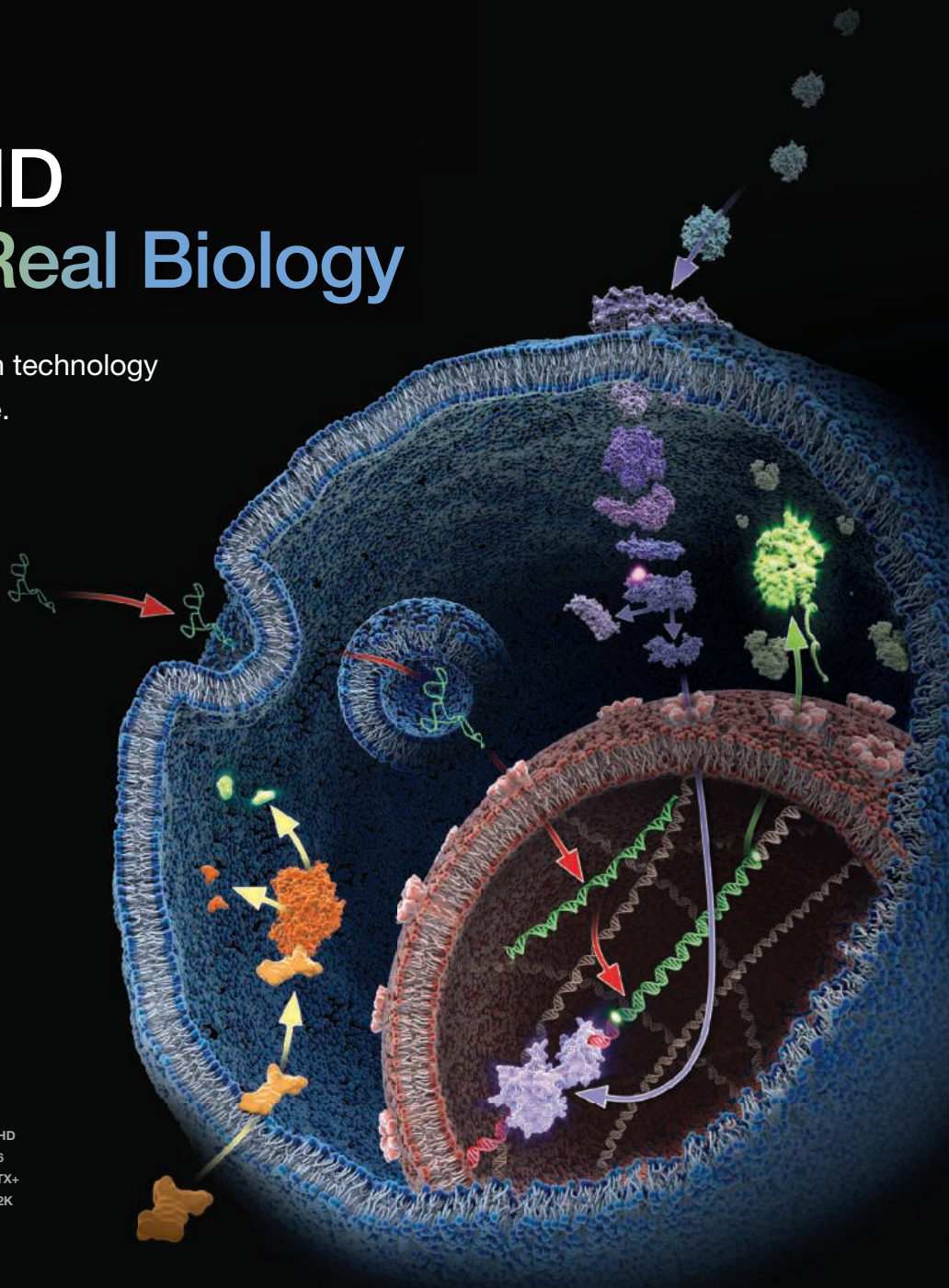
Don't let old toxic transfection technology get in the way of your science. FuGENE[®] HD provides the power to transfect virtually any cell type while maintaining biologically relevant cell signaling responses.

Key applications:

- Cancer Biology
- Stem Cell Research
- Developmental Biology
- Neurobiology
- Immunobiology
- Lentivirus Production
- CHO Cell Protein Production



In this BaF3 cell model of NF-κB luciferase induction, only FuGENE[®] HD and FuGENE[®] 6 are able to create a usable assay.



To discover the power of FuGENE[®] HD for your biology, request a free sample at www.promega.com/pathwaybiology

There's only one

Science



Science Careers Advertising

For full advertising details, go to ScienceCareers.org and click For Employers, or call one of our representatives.

Tracy Holmes

Worldwide Associate Director
Science Careers
Phone: +44 (0) 1223 326525

THE AMERICAS

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

Tina Burks

East Coast/West Coast/South America
Phone: 202-326-6577

Marci Gallun

Midwest/Canada
Phone: 202-326-6582

Candice Nulsen

Corporate
Phone: 202-256-1528

Online Job Posting Questions

Phone: 202-312-6375

EUROPE / INDIA / AUSTRALIA / NEW ZEALAND / REST OF WORLD

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223 326532

Axel Gesatzki

Phone: +44 (0)1223 326529

Sarah Lelarge

Phone: +44 (0) 1223 326527

Kelly Grace

Phone: +44 (0) 1223 326528

JAPAN

Yuri Kobayashi

Phone: +81-(0)90-9110-1719
E-mail: ykobayas@aaas.org

CHINA / KOREA / SINGAPORE / TAIWAN / THAILAND

Ruolei Wu

Phone: +86-1367-1015-294
E-mail: rwu@aaas.org

All ads submitted for publication must comply with applicable U.S. and non-U.S. laws. Science reserves the right to refuse any advertisement at its sole discretion for any reason, including without limitation for offensive language or inappropriate content, and all advertising is subject to publisher approval. Science encourages our readers to alert us to any ads that they feel may be discriminatory or offensive.

Science Careers

From the journal Science



ScienceCareers.org



UNIVERSITY OF
NOTRE DAME

Faculty Positions in Analytical Sciences and Engineering

The University of Notre Dame announces six new faculty positions in Analytical Sciences and Engineering. The positions range from junior level through tenured senior faculty to endowed chair appointments. Successful candidates may choose to establish their programs in Notre Dame's Department of Chemistry and Biochemistry or the Department of Chemical and Biomolecular Engineering, or both.

Since 2006, Notre Dame has initiated the fastest growing analytical sciences program in the U.S., and now seeks to build upon that momentum by leveraging the initial investment in excellence with a disciplined hiring program designed to attract world-leading scholars in the chemical measurement sciences. These positions will impact the analytical sciences by taking advantage of parallel efforts in the targeted departments, the university, the region, and the state of Indiana - including: (a) strongly overlapping hiring initiatives in Notre Dame's Colleges of Science and Engineering, (b) investments in molecular diagnostics through the Advanced Diagnostics & Therapeutics strategic research investments, (c) initiatives to establish degree programs in analytical sciences, and (d) state-wide education and research initiatives developed in concert with colleagues at Indiana University and Purdue University.

The positions include salary, start-up funding and laboratory space commensurate with level. Junior candidates should include a cover letter, curriculum vitae, detailed research plan, and statement of teaching interests directed to Paul W. Bohn/Norman J. Dovichi, Chair, ASEND Search Committee (<https://academicjobsonline.org/ajob/3091>). Candidates must also arrange to have at least three letters of recommendation sent to the search committee via the application website, although senior applicants can apply in confidence. Review of applications will commence immediately and will continue until suitable candidates are identified.

The University of Notre Dame is an Equal Opportunity Employer with a strong institutional commitment to diversity and endeavors to foster a vibrant learning community animated by the Catholic intellectual tradition.



Senior Faculty Position

The Center for Immunology & Microbial Disease at Albany Medical College invites applications for a tenure-track Associate/Full Professor position from individuals with demonstrated research productivity and interest in translational research. Applicants for this senior faculty position should have an MD, MD/PhD or PhD, and lead an internationally-recognized research program in immunology and/or host-pathogen interactions with particular emphasis on disease mechanisms in humans. Successful candidates will also demonstrate strong communication skills and an ability to excel in collaborative research endeavors. The position will have a critical role in the development of joint research programs between the Interdisciplinary Research Center in Immunology and Microbial Disease and the Departments of Internal Medicine and Pediatrics. This position will also have broad responsibility for leading a program for training of doctoral and physician research scientists emphasizing host-pathogen interactions. Joint appointments in divisions of clinical interest are encouraged. The CIMD faculty comprises a highly collaborative research group that this year received \$4.6M in NIH funding, ranking it within the top half of all Microbiology and Immunology programs in the country. The successful candidate will receive an attractive start-up package including the ability to recruit and lead a focused group to be located in our newly constructed 4500 sq ft dedicated laboratory space. This new facility has access to all departmental core services including the Center's fully-staffed Immunology and ABSL-3/BSL-3 Cores. We also have a cooperative program with Translational Medicine that includes use of a new Clinical Research Unit capable of performing both investigator-initiated and industry-sponsored Phase 1/2 infectious disease clinical trials. Albany Medical College is located in a mid-sized city within the upstate New York Capital Region, and has easy access to Boston, New York City, and the Adirondack Mountains.

Applicants should send their curriculum vitae, a statement of research plans, and contact information for three references to:

Faculty Search Committee
Center for Immunology & Microbial Disease and
Infectious Diseases Program, Department of Internal Medicine
Albany Medical College
47 New Scotland Avenue, MC-151
Albany, NY 12208

For further information about the Center, visit www.amc.edu/Research/IMD/

AMC supports a diversified, smoke-free environment and is proud to be an Equal Opportunity/Affirmative Action Employer, encouraging women and minorities to apply. In support of a safe, drug-free environment, criminal background checks and drug testing are part of our hiring process.

DISTINCTIVE LEADERSHIP AND RESEARCH OPPORTUNITY

with one of the world's fastest-rising
universities. **NTU, Singapore**



Nanyang Technological University, Singapore, invites applications for the position of **DEAN, COLLEGE OF ENGINEERING**

About the Appointment

Nanyang Technological University is seeking an accomplished and visionary academic and research leader for the position of the Dean of the College of Engineering.

Reporting to the Provost, the Dean will provide foundational vision, leadership and oversight for the strategic, academic and intellectual affairs of the College and its six constituent Schools. The Dean is expected to lead on an inter-disciplinary basis within and amongst Colleges, and build the human capital of the Engineering College with proactive recruitment of world-class faculty. Other critical responsibilities include cultivating areas of academic and research excellence including inter-College collaboration, providing leadership for securing external research funding support, and interaction with external stakeholders to raise the College's profile, academic standing and engagement. The Dean is also a member of the University Cabinet, the highest management decision-making body, and other senior leadership committees at the University.

This is an opportunity for an outstanding academician who is passionate about inter-disciplinary teaching and research, and about growing and strengthening a dynamic College, in a collaborative manner with other Colleges, within a University with a high global impact.

The appointee must have an outstanding record of academic leadership, research and teaching in a reputable university or academic institution. The appointee must demonstrate leadership, vision, and outstanding interpersonal skills to engage faculty and administrators across campus. Other essential attributes include the ability to effectively communicate with stakeholders, work cooperatively with national funding agencies and commit to faculty-shared governance. The appointee must also be passionate and committed to enhancing the existing academic strengths of the College in research, scholarship and education.

About Singapore

Singapore is a dynamic centre for the pursuit of science, technology, and innovation, and the vibrant city-state is known for its commitment to academic excellence and research. Singapore aims to be a global centre for academic excellence in higher education and research, and this strategic goal and direction has been supported by major public sector investments. Uniquely situated in an emergent and vibrant Asia, Singapore combines the eastern and western approaches to governance, education, and lifestyle. Having forged lasting and synergistic business and academic relationships with China, India, other Asian countries and beyond, Singapore sits at the crossroads of cultures and peoples.

About the University

Nanyang Technological University (NTU) is an internationally renowned research-intensive university with globally acknowledged strengths in Engineering and Business. Science and Humanities have added to the strengths of the University, and a Medical School has been set up jointly with Imperial College London. Ranked 8th in Asia and 41st in the 2013 Quacquarelli Symonds (QS) World University Rankings, NTU is the fastest rising Asian university in the QS Top 50. In this ranking, NTU's Engineering and Technology is ranked 14th in the world and 3rd in Asia. NTU is also the 2nd in the world among the young elite universities in the QS' rankings.

NTU is the 5th most-cited university for engineering research output that is among the top three universities globally (Essential Science Indicators, January 2013). The Electrical and Electronic Engineering and Computer Engineering schools have both been ranked 1st in Singapore and 4th in the world (after MIT, University of California, Berkeley, and Stanford University) by the Higher Education Evaluation & Accreditation Council of Taiwan. In the QS World University Rankings by Subject 2013, NTU is ranked within the world's top 20 for Civil Engineering (8th worldwide and first in Singapore), Mechanical Engineering (11th), Communication & Media Studies (11th), Education (13th), Electrical Engineering (14th) and Materials Science (14th). NTU was placed 32nd globally and 6th in Asia in the 2013 Financial Times Global MBA Rankings. Its accounting research in the business school has been ranked 1st in Asia and 5th position globally in the latest Brigham Young University (BYU) Accounting Research Rankings.

The University's academic and research programmes, with real-world relevance, have received strong support from major corporations and industry, in terms of research funding, industry partnerships, and global internship opportunities for the students.

A founding member of the Global Alliance of Technological Universities, NTU aims to groom active citizens of the world who can lead and manage new, complex, global challenges. The University provides a high-quality comprehensive and global education to more than 23,500 undergraduates and 9,500 graduate students. Together with the University's 3,800-strong faculty and research staff who bring international academic perspectives and depth of experience, the University's main 200-hectare residential garden campus – located at the south-western part of Singapore – is a hub for vibrant academic endeavours.

About the College

NTU's four colleges – College of Engineering; College of Science; College of Business (Nanyang Business School); and College of Humanities, Arts, and Social Sciences – comprise 12 component schools. The College of Engineering is one of the largest engineering colleges in the world, housing six constituent Schools, 14,000 students, 600 faculty and 1,600 staff. The six schools are the School of Chemical and Biomedical Engineering, the School of Civil and Environmental Engineering, the School of Computer Engineering, the School of Electrical and Electronic Engineering, the School of Materials Science and Engineering and the School of Mechanical and Aerospace Engineering.

More information on the College can be accessed at: <http://coe.ntu.edu.sg>

To apply, please send your curriculum vitae, accompanied by a cover letter, to:

**The Vice-Chairman of the Search Committee, Professor S. Shankar Sastry
Dean, College of Engineering**

**Roy W. Carlson Professor of Electrical Engineering and Computer Science, Bioengineering & Mechanical Engineering
University of California, Berkeley**

**c/o Secretary to the Search Committee
Nanyang Technological University, Level 4, Administration Building
50, Nanyang Avenue, Singapore 639798
Fax: (65) 6795 9001 Email: DEANSEARCHCOE@NTU.EDU.SG**

Applications will be welcomed up to 15 January 2014

Korn/Ferry International Pte Ltd is the appointed Executive Search firm for the Dean Search.

All applications and materials submitted will be held in strict confidence.



OPEN FACULTY POSITIONS INSTITUTE OF MOLECULAR BIOLOGY ACADEMIA SINICA, TAIWAN, ROC

One tenure-track faculty position is open for a highly qualified individual to establish independent research programs in **all disciplines of molecular, cellular, and systems biology**. Applicants should hold a Ph.D. degree or its equivalent, and postdoctoral research experience is preferred. The successful recruit will be appointed at the levels of **Assistant, Associate, or Full Research Fellows** (equivalent to academic ranks of Assistant, Associate and Full Professors at universities), and receive a generous multi-year start-up package, followed by annual intramural support.

The Institute of Molecular Biology at Academia Sinica (<http://www.imb.sinica.edu.tw/en>) provides an active and stimulating research environment, is well supported by both extramural and long-term intramural funding, and features several core facilities (imaging, microarray, Next Generation Sequencing, RNAi, electrophysiology, FACS, bioinformatics and mouse facilities) that provide state-of-the-art resources and key technical expertise to the Institute's research community. Recent research works were published in top journals such as Science, Nature, and Cell. Currently three Ph.D. programs, with one recruiting international students, are formally affiliated with the Institute. English is the official language for regular seminars and most of the lectures at the Institute, and proficiency in Chinese language is not a prerequisite for application.

Applicants should send their Curriculum Vitae, a description of past research accomplishments and future research plans, and arrange for three letters of recommendation to be sent directly to:

Dr. Soo-Chen Cheng, Director
c/o Ms. Vivi Chiang
Institute of Molecular Biology,
Academia Sinica
Taipei, Taiwan 11529, ROC

The selection process will start on **December 15, 2013** until the positions are filled. Further information can be obtained from **Ms. Vivi Chiang** at vivi@imb.sinica.edu.tw



Bioinformatics/Computational Biology

The Center for Bioinformatics (Bioinformatics Program) and the Department of Molecular Biosciences invite applications for an assistant professor tenure-track faculty position to begin as early as August 18, 2014. The interdisciplinary Center for Bioinformatics (www.bioinformatics.ku.edu) complements existing strengths in the Department of Molecular Biosciences (www.molecularbiosciences.ku.edu), including structural biology, computational chemistry, proteomics, systems biology, and developmental/molecular genetics, as well as strengths in drug design and information technology in the Schools of Pharmacy and Engineering. The Center fosters international activities in Bioinformatics and combines outstanding research and a Ph.D. program.

Required Qualifications: Ph.D. and postdoctoral experience in a discipline related to Bioinformatics is expected by the start date of the appointment; potential for excellence in research in Bioinformatics; commitment to teaching life sciences courses; and strong record of research accomplishments in at least one of the following areas: modeling of macromolecular structure and interactions, modeling of protein networks, systems biology, genomics, chemical biology, and computer-aided drug discovery.

For the full position announcement and to apply online, go to: <http://employment.ku.edu> and Search Faculty Job openings, key words "Bioinformatics/Computational Biology". Submit a CV, letter of application, statement of past and future research, statement of teaching interests and philosophy, and a list of at least three references who may be contacted via telephone or e-mail. Initial review of applications begins **November 1, 2013** and will continue as long as needed to identify a qualified pool. Direct inquiries to **Dr. Ilya Vakser** (vakser@ku.edu).

The University of Kansas is especially interested in hiring faculty members who can contribute to four key campus-wide strategic initiatives: (1) Sustaining the Planet, Powering the World; (2) Promoting Well-Being, Finding Cures; (3) Harnessing Information, Multiplying Knowledge; and (4) Building Communities, Expanding Opportunities. See www.provost.ku.edu/planning/themes/ for more information. Equal Opportunity Employer M/F/D/V.



IUPUI

Biology Tenure Track Faculty

The Department of Biology (biology.iupui.edu) at Indiana University - Purdue University Indianapolis (IUPUI) invites applications for **two tenure-track faculty positions** to begin August 1, 2014. Rank is open and competitive salary and start-up funds are available. A Ph.D. and postdoctoral experience are required. Applicants must demonstrate the ability to initiate and sustain an externally funded program of research, dedication to effective student mentoring and the ability to teach courses at the graduate and undergraduate levels. Candidates able to contribute to teaching in genetics, molecular biology, biochemistry and bioinformatics are particularly welcome. Research strengths in the department span a range of topics in cellular, molecular, developmental and regenerative biology. Applications from individuals whose research expertise encompasses computational/bioinformatics approaches are encouraged. Applicants at a senior level must have an established record of research excellence and external funding, and a demonstrated commitment to mentoring students.

Applicants should submit one PDF file to BIOLIFAC@iupui.edu including a cover letter, curriculum vitae, and statement of research and teaching interests/experience. Candidates should also arrange to have three letters of recommendation sent to the same address. Evaluation of completed applications will begin **November 1st, 2013** and continue until the position is filled. The IUPUI campus, located in downtown Indianapolis, has a student population in excess of 30,000 and attracts more than \$300 million in annual research funding. The Department of Biology has a strong record of externally funded research and training of Ph.D. students. The department occupies well-equipped research laboratories and core facilities, which will be significantly expanded with the completion of a new research building this year. With an extensive hospital system and the IU School of Medicine, IUPUI is home to a large and vibrant community of researchers in all areas of biomedical science.

IUPUI is an EEO/AA Employer; M/F/D.

UNIVERSITY of CALIFORNIA · IRVINE

Faculty Positions in Pharmaceutical Sciences

Assistant Professor: The Department of Pharmaceutical Sciences invites applications for a tenure track faculty position in the field of pharmacogenomics. The appointment will likely be made at the Assistant Professor level, but tenured Associate or Full Professor appointments may be considered for exceptionally well qualified applicants with the appropriate experience.

The successful candidate will be expected to establish a world-class research program in the area of pharmacogenomics, broadly defined. The candidate should also be committed to teach subjects related to pharmaceutical sciences at undergraduate and graduate levels. We anticipate hiring an individual who is looking for a collegial and interdisciplinary environment with numerous opportunities for establishing collaborations with research groups in the department, the School of Medicine, the UCI Comprehensive Cancer Center, and many other allied research programs at UCI. **To apply go to <https://recruit.ap.uci.edu/apply/JPF02101>**

Lecturer, PSOE: The Department of Pharmaceutical Sciences invites applications for a Lecturer position with potential for security of employment. This position parallels the tenure track research faculty series at the level equivalent to assistant professor, although applications at higher levels will be considered. The major focus in this position will be on developing and coordinating effective curricula and instructional activities in both lower and upper division undergraduate laboratory and lecture courses. We seek outstanding Ph.D.s with innovative ideas for instructional initiatives in molecular pharmacology, pharmaceuticals, medicinal chemistry, toxicology, and/or any of the other disciplines in the pharmaceutical sciences. **To apply go to <https://recruit.ap.uci.edu/apply/JPF02100>**

Review of applications for both positions will begin **November 15, 2013**. To ensure full consideration, applications and all supporting materials should be received by that date. The position will remain open until filled.

The University of California is an Equal Opportunity/Affirmative Action Employer committed to excellence through diversity, and has an active ADVANCE program dedicated to gender and ethnic equity.

UC DAVIS

**Assistant Professor in Crop Ecology/Agroecology
Department of Plant Sciences
University of California, Davis**

RESPONSIBILITIES: The successful candidate's research will focus on crop production in agricultural systems. Possible research topics include improving resource use efficiency in diverse cropping systems, alternative approaches to attain sustainable production of annual and perennial crops, and utilizing ecologically-based approaches to reduce external inputs while maintaining crop yields. Interest in California agriculture is necessary and in international agriculture is desirable. Teaching responsibilities will involve a major role in the Sustainable Agriculture and Food Systems undergraduate major and include courses in sustainable agriculture systems, agroecosystem management, and crop ecology. Graduate courses will be part of the Horticulture and Agronomy and/or Ecology Graduate Groups. Advising and mentoring of undergraduate and graduate students is expected. The position is an academic year (9 month) tenure-track position. This Assistant Professor position will include an appointment in the Agricultural Experiment Station. Faculty members who hold an Agricultural Experiment Station appointment have a responsibility to conduct research and outreach relevant to the mission of the California Agricultural Experiment Station. It is anticipated that the candidate will collaborate with other scientists at UC Davis including staff and faculty affiliates of the Agricultural Sustainability Institute, Cooperative Extension specialists, farm advisors, and researchers from other universities and agencies to address the mission of the Department, College, and Agricultural Experiment Station. The successful candidate will be expected to participate in departmental, college, and campus committees, and with state, regional and national organizations, as appropriate.

QUALIFICATIONS: Candidates must have a strong and well-documented background in agroecology, crop ecology, agroecosystem management, agronomy, or related fields and a Ph.D. or equivalent degree in an appropriate discipline. Candidates must have the ability to conduct independent and cooperative research, and a willingness to address research areas relevant to the mission of the Agricultural Experiment Station.

SALARY: Commensurate with qualifications and experience.

TO APPLY: Candidates should begin the application process by registering online at <http://recruitments.plantsciences.ucdavis.edu>. Please include statements of research and teaching interests, *curriculum vitae*, publication list, copies of 3 of your most important research publications, copies of undergraduate and graduate transcripts (if within 5 years of either degree), and the names, e-mail addresses, and telephone numbers of at least five professional references. For technical or administrative questions regarding the application process, please email mjgreenleaf@ucdavis.edu. Review of the applications will begin **November 1, 2013**. The position will remain open until filled.

Dr. Arnold Bloom, Chair, Search Committee

Department of Plant Sciences

University of California

One Shields Avenue

Davis, CA 95616-8515

Telephone: (530) 752-1743 / FAX: (530) 752-9659

E-mail: ajbloom@ucdavis.edu

The University of California, Davis, and the Department of Plant Sciences are interested in candidates who are committed to the highest standards of scholarship and professional activities, and to the development of a campus climate that supports equality and diversity. The University of California is an Affirmative Action/Equal Opportunity Employer.

THE UNIVERSITY OF HONG KONG



Founded in 1911, The University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. The University has a comprehensive range of study programmes and research disciplines spread across 10 faculties and about 110 sub-divisions of studies and learning. There are over 27,800 undergraduate and postgraduate students coming from 50 countries, and more than 2,000 members of academic and academic-related staff, many of whom are internationally renowned.

Post-doctoral Fellowships and Research Assistant Professorships

Applications are invited for a number of positions as Post-doctoral Fellow (PDF) and Research Assistant Professor (RAP), at the University of Hong Kong, on or before July 31, 2014. Appointments will be made for a period of 2 to 3 years.

PDF and RAP posts are created specifically to bring new impetus and vigour to the University's research enterprise. Positions are available from time to time to meet the strategic research needs identified by the University. Positions are available in the following Faculties/Departments/Schools/Centres:

- Faculty of Dentistry
- Faculty of Education
- Civil Engineering
- Computer Science
- Electrical and Electronic Engineering
- Mechanical Engineering
- Law
- Biochemistry
- Clinical Oncology
- Centre for Cancer Research
- Research Centre of Heart, Brain, Hormone and Healthy Aging
- Centre for Genomic Sciences
- Research Centre of Infection and Immunology
- Orthopaedics and Traumatology
- Physiology
- Centre for Reproduction, Development and Growth
- Stem Cell and Regenerative Medicine Consortium
- School of Biological Sciences
- Chemistry
- Geography
- Social Work and Social Administration
- The State Key Laboratory of Emerging Infectious Diseases
- The State Key Laboratory for Liver Research

Post-doctoral Fellows

PDFs are expected to devote full-time to research. Applicants should be doctoral degree holders having undertaken original research that has contributed to the body of knowledge. A highly competitive salary commensurate with qualifications and experience will be offered. Annual leave and medical benefits will also be available.

Research Assistant Professors

The main focus of an RAP's duty is research. RAPs can however be assigned some teaching duties, up to 50% of the normal teaching load. Applicants should be research active and have a proven publication record. A highly competitive salary commensurate with qualifications and experience will be offered, with a contract-end gratuity and University contribution to a retirement benefits scheme (totalling up to 15% of basic salary). Annual leave and medical benefits will also be offered.

Procedures

Prospective applicants are invited to visit our webpage at <http://jobs.hku.hk> to view the list of the Faculties/Departments/Schools/Centres and their research areas for which PDF/RAP positions are currently available. Before preparing an application, they should contact the Head of the appropriate academic unit to ascertain that their research expertise matches the research area for which a vacant PDF/RAP post is available.

Applicants must submit a completed University application form, which should clearly state **which position** they are applying for; and in which academic discipline. They should also provide further information such as details of their research experience, publications, research proposals, etc.

Application forms (341/1111) can be obtained at <http://www.hku.hk/apptunit/form-ext.doc>. Further particulars can be obtained at <http://jobs.hku.hk/>. **Closes October 28, 2013.** The University thanks applicants for their interest, but advises that only shortlisted applicants will be notified of the application result.

The University is an equal opportunity employer and is committed to a No-Smoking Policy



UCI IRVINE | UNIVERSITY
of CALIFORNIA

Neuroscience Faculty Recruitment Anatomy and Neurobiology, University of California, Irvine

The Department of Anatomy and Neurobiology, School of Medicine at the University of California Irvine seeks exceptional applicants for a tenure track appointment in neuroscience. The successful applicant is expected to develop a dynamic research group to study the function, plasticity or development of **Neuronal Circuits**. Candidates with interests in in vivo reversible manipulations of defined neuronal subpopulations, models of neurological or psychiatric disorders, and/or combined experimental and computational modeling approaches are especially encouraged to apply. A strong publication record and ability to develop and maintain an independently funded research program is essential. Although emphasis will be placed on recruiting an Assistant Professor, excellent candidates at the Associate or Full Professor levels will also be seriously considered. All applicants must hold a PhD, MD or an MD/PhD degree, have an outstanding record of published research, and a commitment to teaching in medical and graduate courses.

The University of California Irvine has an exceptionally strong and broad program in the neurosciences and aims to enhance interactions between basic and clinical research, and to link the neurosciences with other scientific disciplines within the University. New faculty will be affiliated with the Department of Anatomy and Neurobiology in the School of Medicine and with the campus-wide Interdepartmental Program in Neurobiology.

Applications should be submitted prior to **December 1, 2013**, through UC Irvine's RECRUIT system located at <https://recruit.ap.uci.edu/apply/JPF02114>. Applicants should complete the online application profile and upload the following application materials electronically to be considered for the position: 1. Cover letter, including a two-page summary of research interests; 2. Curriculum vitae; 3. Names and email addresses of at least 3 individuals who can provide letters of reference.

UCI is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity.



The University of Texas at Dallas
School of Natural Sciences
and Mathematics

FACULTY POSITIONS IN THE DEPARTMENT OF MOLECULAR AND CELL BIOLOGY

The Department of Molecular and Cell Biology in the School of Natural Sciences and Mathematics at The University of Texas at Dallas (<http://www.utdallas.edu/biology/>) invites applications for Faculty positions at any rank. Preference will be for investigators working in research areas that complement existing Departmental strengths in biochemistry, cancer biology, cell biology, computational and systems biology, microbiology, and neurobiology. The Department will participate in a new School-wide interdisciplinary initiative in basic energy sciences. Researchers addressing fundamental questions that complement our strengths and that relate to bioenergy are especially encouraged to apply. Applicants should show evidence of a vigorous and independent research program, and those at the Associate or Full Professor levels should also have a record of obtaining significant external funding. Applicants should have enthusiasm for teaching at both graduate and undergraduate levels. Review of applications will begin immediately and will continue until the positions are filled. Indication of sex and ethnicity for affirmative action statistical purposes is requested as part of the application but is not required. Application materials (curriculum vitae, short descriptions of research plans and teaching interests, and three letters of recommendation) should be submitted via the ONLINE APPLICATION available at <http://go.utdallas.edu/pnc130907>

The University of Texas at Dallas is an Equal Opportunity/Affirmative Action Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability, pregnancy, age, veteran status, genetic information or sexual orientation.

Genomic Approaches to the Study of Gene Regulation

Cornell is a community of scholars, known for intellectual rigor and engaged in deep and broad research, teaching tomorrow's thought leaders to think, care for others, and create and disseminate knowledge with a public purpose.

The Department of Molecular Biology and Genetics, Cornell University, invites applications for a tenure-track faculty position at the Assistant Professor level. The ideal candidate will apply both experimental and bioinformatics approaches to study gene regulation. Individuals seeking a creative integration of experimental, computational and comparative approaches will find Cornell a particularly rich environment in which to work (see <http://mbg.cornell.edu/cals/mbg/mbg-search.cfm>). An advanced degree (Ph.D., M.D., or equivalent) is required and postgraduate training is highly desirable. Candidates should submit to <https://academicjobsonline.org/ajo/jobs/3254>, a CV, a two to four page research statement, which indicates how your program could synergize with existing Cornell faculty, a one page teaching statement, and PDFs of two of your most significant papers. Application review begins on November 15, 2013.

Find us online at <http://hr.cornell.edu/jobs> or
[Facebook.com/CornellCareers](https://www.facebook.com/CornellCareers)

Cornell University is an innovative Ivy League university and a great place to work. Our inclusive community of scholars, students and staff impart an uncommon sense of larger purpose and contribute creative ideas to further the university's mission of teaching, discovery and engagement. Located in Ithaca, NY, Cornell's far-flung global presence includes the medical college's campuses on the Upper East Side of Manhattan and in Doha, Qatar, as well as the new CornellNYC Tech campus to be built on Roosevelt Island in the heart of New York City.



Diversity and inclusion have been and continue to be a part of our heritage. Cornell University is a recognized EEO/AA employer and educator.



UNC
LINEBERGER

Faculty Positions in Basic Translational Sciences

The UNC Lineberger Comprehensive Cancer Center, in collaboration with departments in the School of Medicine and across the entire University of North Carolina Chapel Hill, seeks outstanding candidates for faculty positions at all levels and at all ranks in basic and translational cancer research with a special interest in a senior cancer researcher. This broad-based recruitment seeks outstanding scientists in a number of areas, including but not limited to: animal models, signal transduction, cancer genetics, bioinformatics, virology, drug development and target validation, epigenetics and gene expression, DNA damage and repair, cancer therapy, cancer immunology, inflammation and cancer, and stem cells. Applicants should have a strong record of recent accomplishments as a post-doctoral fellow or sustained productivity as an established faculty member. Appointment and rank in an academic department will be determined by the applicant's qualifications.

Applications will be reviewed beginning December 1, 2013 and until the positions are filled.

Educational Requirements: Doctoral Degree
Qualifications and Experience: Doctoral Degree

Apply online at <http://unc.peopleadmin.com/postings/31918> and provide curriculum vitae, a list of four references, and Research Statement.

The University of North Carolina at Chapel Hill is an Equal Opportunity Employer. Women and minorities are strongly encouraged to apply and self-identify on their application.

CAREER TRENDS Running Your Lab



Download your free copy today at
ScienceCareers.org/booklets

Science Careers
From the journal *Science* AAAS

Brought to you by the
AAAS/Science Business Office

 **UMASS AMHERST**

Assistant Professor of Microbiology

The Department of Microbiology invites applications from Ph.D.-level scientists for a tenure-track position at the level of ASSISTANT PROFESSOR. The Department's 15 faculty members and affiliated units at the University have broad research strengths in microbiology, immunology, immunity and host defense, microbial pathogenesis, virology, microbial physiology, genetics, genomics, environmental microbiology, and biotechnology. We are seeking outstanding candidates taking innovative, molecular and cellular approaches to the study of Medical Virology. This area is broadly defined and would include the study of viral, prokaryotic and or eukaryotic and prionic pathogens. The successful candidate will have had at least three years postdoctoral experience and will have published several articles in high impact peer-reviewed journals. He/she will be expected to establish a strong independent, extramurally funded research program and participate in the teaching of undergraduate and graduate courses. Research facilities that include new animal care and BSL III facilities, and competitive salary and start-up funds will be provided. Opportunities exist to establish strong collaborations with faculty in the Five College Area and at Bay State Medical Center.

Applicants should send a curriculum vitae, a statement of research and teaching interests, reprints of recent publications, and at least three letters of recommendation to: Chair of Microbiology Search Committee R45589, Department of Microbiology, University of Massachusetts, N203 Morrill IV North, Amherst, MA 01003, microbio-dept@microbio.umass.edu. The search committee will begin reviewing applications on October 21, 2013, and will continue until the position is filled. Hiring is contingent upon the availability of funds.

The University seeks to increase the diversity of its professoriate, workforce and undergraduate and graduate student populations because broad diversity is critical to achieving the University's mission of excellence in education, research, educational access and service in an increasingly diverse globalized society. Therefore, in holistically assessing many qualifications of each applicant of any race or gender we would factor favorably an individual's record of conduct that includes students and colleagues with broadly diverse perspectives, experiences and backgrounds in educational, research or other work activities. Among other qualifications, we would also factor favorably experience overcoming or helping others overcome barriers to an academic career or degree. The University of Massachusetts Amherst is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.

 **NATIONWIDE CHILDREN'S**
When your child needs a hospital, everything matters.™

 **THE OHIO STATE UNIVERSITY**

FACULTY POSITIONS CENTER FOR VACCINES AND IMMUNITY

The CENTER FOR VACCINES AND IMMUNITY of The Research Institute at Nationwide Children's Hospital and The Ohio State University Department of Pediatrics is seeking PhD, MD or MD/PhD candidates for tenure-track positions at the Assistant, Associate or Professor level. Successful candidates will have an established, independent research program on virus infections with a translational component related to human disease. Specific areas of interest include viruses that affect the respiratory and GI tracts, hepatitis and cytomegalovirus infections. Candidates studying the innate or adaptive immune response to these infections or to polymicrobial respiratory tract infections are particularly encouraged to apply. Research on viral infections in the Center for Vaccines and Immunity is supported by a close working relationship with the Infectious Diseases Division of Nationwide Children's Hospital including access to large patient populations for translational studies and a special infectious diseases unit dedicated to facilitating patient identification and recruitment. The Center has a facility that produces primary well differentiated human airway epithelial cultures. The Institute has an AAALAC accredited vivarium with expertise in animal infection, particularly rodent and nonhuman primates, and provides state-of-the-art, subsidized core services for flow cytometry, bioinformatics, morphology, viral vector production, genomics, ES cell, transgenic and small animal imaging. The OSU campus provides many other cores and opportunities for collaboration.

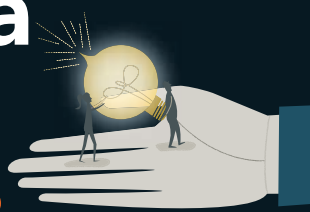
Generous start-up support and joint appointments within OSU graduate departments and with clinical divisions at Nationwide Children's Hospital are available. For more information, please visit our website at <http://www.nationwidechildrens.org/center-for-vaccines-and-immunity>

The Ohio State University is an Equal Opportunity, Affirmative Action Employer. Women, minorities, veterans, and individuals with disabilities are encouraged to apply.

Send curriculum vitae with contact information for three references to:
Mark.Peebles@nationwidechildrens.org

Join the Minds of Janelia Lab Heads

Janelia Farm Research Campus



An extraordinary scientific opportunity

Janelia Farm Research Campus, a pioneering biomedical research complex, is recruiting researchers from diverse disciplines to explore questions in basic neuroscience, imaging technology, and related fields of research.

Highlights

- Access to unparalleled scientific and technical facilities
- Internally funded, requiring no extramural grants
- No formal teaching responsibilities
- Minimal administrative duties

Application deadline:
2PM ET November 15, 2013

www.janelia.org/sci

HHMI
janelia farm
research campus

POSITIONS OPEN

TENURE TRACK FACULTY POSITION in Structural Biology The University of Kansas Medical Center

The Department of Biochemistry and Molecular Biology at the University of Kansas Medical Center ([website: http://www.kumc.edu/biochemistry](http://www.kumc.edu/biochemistry)) invites applications for a tenure-track faculty position at any rank, with a start date as early as January 1, 2014. We are interested in candidates who incorporate X-ray crystallographic approaches to understand structure-function relationships of macromolecules in the context of projects related to human health and disease. Applicants whose research program is relevant to cancer biology, developmental biology, or membrane proteins are especially encouraged to apply. We house a newly remodeled X-ray facility that is equipped with Rigaku/MSO equipment, including an R-Axis IV++ image plate detector with 2-theta stage; Osmic Blue Confocal mirrors; RU-H3R rotating anode X-Ray generator (Cu-K α); and an X-stream 2000 cryogenic system. The facility also includes a large, dedicated wet laboratory and cold room exclusively for the crystallography laboratory. The crystallographer is expected to contribute to the department's mission of graduate and medical student education, and to maintain an independent, vigorous, research program. Interested individuals should submit a single PDF file to **e-mail: xraybmb@kumc.edu** containing the following: a cover letter describing their interest in this position, curriculum vitae, a two-page description of future research plans, and the names and e-mail addresses of three references. Review of Applications will begin on October 15, 2013 and will continue until the position is filled.

The University of Kansas Medical Center is an Equal Opportunity/Affirmative Action Employer.

TENURE-TRACK FACULTY POSITION University of Kansas Medical Center

The Department of Biochemistry and Molecular Biology ([website: http://www.kumc.edu/biochemistry](http://www.kumc.edu/biochemistry)) invites applications from scientists working in the fields of molecular biology, biochemistry, developmental, or cancer biology. The level of appointment is open. Of special interest are applicants with expertise in metabolism, particularly as it relates to cancer or development, although all outstanding applicants will be considered. The faculty member will be expected to establish and maintain an independent, externally funded research program and to participate in the teaching activities of the department, in particular the teaching of metabolism. Current areas of research in this highly interactive medical center include molecular, cell, developmental and cancer biology, neuroscience, hepatic and renal biology, and proteomics. Available departmental and institutional facilities include genomics, transgenic mouse and ES cell gene targeting, confocal, EM and laser capture microscopy, proteomics, and state-of-the-art animal care. Applicants should send curriculum vitae, a statement of research interests and long-range goals, and the names and e-mail addresses of three references to **e-mail: metbmb@kumc.edu**. Review of applications will be begun immediately and will continue until the position is filled.

The University of Kansas Medical Center is an Equal Opportunity/Affirmative Action Employer.

YALE UNIVERSITY Department of Chemistry

The Department of Chemistry, Yale University, New Haven, Connecticut invites applications for multiple tenure-track positions at the **ASSISTANT PROFESSOR** level to commence 1 July 2014. We seek creative teacher-scholars who show promise for developing outstanding research programs and we will consider applications in any area of chemistry. Applicants should send their curriculum vitae, a statement of research plans, and arrange for the submission of three letters of recommendation. Please submit all material to Academic Jobs Online at [website: https://academicjobsonline.org/ajo/Yale](https://academicjobsonline.org/ajo/Yale) position #3156. A review of applications will begin October 1, 2013.

Yale University is an Equal Opportunity/Affirmative Action Employer and applications from women and underrepresented minority group members are especially encouraged.

POSITIONS OPEN

THE UNIVERSITY OF TEXAS AT TYLER Department of Biology

The Department of Biology at The University of Texas at Tyler invites applications for three tenure-track **ASSISTANT PROFESSOR** positions. Doctorate and postdoctoral experience required for all three positions.

i. **Developmental Biology.** Applicants with expertise using model systems to study epigenetics, stem cell biology, evolutionary developmental biology, or sub-cellular developmental processes are encouraged to apply.

ii. **Computational Biology.** Applicants with strong computational skills and expertise in bioinformatics, transcriptomics, pharmacogenomics, metagenomics, or systems biology are encouraged to apply.

iii. **Microbiology.** Applicants specializing in genomic approaches to microbe-microbe, microbe-environment, or microbe-host interactions are encouraged to apply.

Curriculum vitae, statements of research interests and teaching philosophy, and reprints of three publications should be sent as one PDF to **Dr. Blake Bextine** at **e-mail: bbextine@uttyler.edu** (developmental biology), **Dr. Lance Williams** at **e-mail: lwilliams@uttyler.edu** (computational biology), and **Dr. Jim Koukl** at **e-mail: jkoulk@uttyler.edu** (microbiology). Three letters of reference should be sent to the same addresses. Screening will begin immediately and continue until a suitable candidate is identified. Applicants subject to background check. *Equal Opportunity Employer/Affirmative Action Employer.* Full description at [website: http://www.uttyler.edu/biology](http://www.uttyler.edu/biology).

TENURE-TRACK FACULTY POSITION Microbiology

The Department of Biology at California State University San Bernardino invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR** in the area of microbiology. The successful applicant will develop an independent research program and is expected to excel in teaching courses related to microbiology, medical microbiology, microbial ecology, or immunology at the undergraduate and M.S. levels. Candidates must have a record of published research and show potential for developing and sustaining independent, externally funded research involving both undergraduate and M.S. students. Candidates must have a Ph.D., preferably in the biological sciences; postdoctoral experience is desirable. Application deadline is 1 November 2013, but applications will be accepted until the position is filled. Submit curriculum vitae, statement of research accomplishments and goals, statement of teaching philosophy, and three letters of reference to: **Dr. David Polcyn, Chair, Department of Biology, Attn: Microbiology Faculty Search, California State University San Bernardino, 5500 University Parkway, San Bernardino, CA 92407; telephone: 909-537-5305.**

ASSISTANT PROFESSOR in Inorganic Chemistry

Georgetown University wishes to recruit a tenure-track Assistant Professor in Inorganic Chemistry to begin fall 2014. Research areas in inorganic chemistry as broadly defined will be considered that complement those of existing faculty in the Department. Research interests related to clean energy, the environment, and sustainability are especially welcome. Candidates must have a Ph.D. degree in chemistry or a closely related field and postdoctoral training or equivalent is desirable. Development of an internationally recognized, externally funded research program and teaching at the undergraduate and graduate levels, are expected. Please send curriculum vitae, description of research plans, and statement of teaching philosophy as one document in PDF format, and arrange for three letters of recommendation to be sent to **e-mail: chemsearch@georgetown.edu**. For full consideration, complete applications should arrive before October 31, 2013. *Georgetown University is an Equal Opportunity/Affirmative Action Employer fully dedicated to achieving a diverse faculty and staff; applications from qualified women and minority candidates are encouraged.*

POSITIONS OPEN

OPEN POSITION: LECTURER with Potential for Security of Employment University of California, Irvine Ecology and Evolutionary Biology

The University of California, Irvine (UCI) Department of Ecology and Evolutionary Biology invites applications for a tenure-track faculty position as a Lecturer with Potential for Security of Employment. This individual will be responsible for developing and teaching courses in ecology and sustainability studies, including those in the Interdisciplinary Minor in Global Sustainability, and for helping to create a new Professional Master's Program in Conservation and Restoration Science. We seek outstanding candidates with a Ph.D. in biology or a related scientific discipline and demonstrated excellence in teaching, course development, and scientific writing. The successful applicant will preferably have training or experience in an ecology-related field, such as ecological restoration, conservation biology, sustainability science, or global change biology.

Applicants should submit the following via **website: http://recruit.uci.edu/** curriculum vitae, a statement of teaching interests, and three letters of reference. Review of applications will begin on November 1, 2013, and the position will remain open until filled. Applications due by: November 1, 2013.

The University of California, Irvine is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity.

ASSISTANT PROFESSOR OF GENOMICS

Genomics Faculty Position. The Department of Biology at Clark University, Worcester, Massachusetts ([website: http://www.clarku.edu/departments/biology/](http://www.clarku.edu/departments/biology/)) invites applications for a tenure-track appointment at the rank of Assistant Professor to begin fall 2014. The successful candidate is expected to develop an externally funded research program in any field of genomics, involving Ph.D., M.S., and undergraduate students, and is likely to teach genetics and courses in their area of expertise. For full consideration, one electronic file with curriculum vitae, research and teaching statements, and three key publications should be received prior to November 1, 2013, as should three letters of reference (**e-mail: genomics@clarku.edu**). See ([website: http://www.clarku.edu/offices/hr](http://www.clarku.edu/offices/hr)) for full position description. Inquiries can be directed to **Susan Foster** (**e-mail: sfoster@clarku.edu**). *Clark University is committed to sustaining a diverse and inclusive community that cultivates ethical and well-informed citizens. We especially welcome candidates who can contribute to this mission through research, teaching, and/or service. Affirmative Action/Equal Opportunity Employer. Minorities and women are strongly encouraged to apply.*

FACULTY POSITIONS in Power Systems and Complex Systems College of Engineering & Mathematical Sciences The University of Vermont

The College of Engineering and Mathematical Sciences invites applications for two tenure-track faculty positions in support of our NSF-funded Smart Grid IGERT program. The first position is in Power Systems, or related areas such as power electronics or control systems. The second position is in Complex Systems, with a focus on research problems in the behavioral, social, or economic sciences. Requirements include an earned doctorate in engineering, computer science, mathematics, or a related discipline, a proven record of scholarly activities, and the qualifications to teach both undergraduate and graduate courses in one of the College's degree programs. The full job description and application guidelines can be found at **website: http://www.cems.uvm.edu/facsearch/igert.php**. The first review of applications will occur on December 1, 2013. *University of Vermont is an Equal Opportunity/Equal Access/Affirmative Action Employer and conducts background checks on all final candidates.*



INSTITUTE OF MEDICINE

OF THE NATIONAL ACADEMIES

Executive Officer

The Institute of Medicine of the National Academies (IOM) seeks an Executive Officer. This is an exceptional opportunity for a dynamic and creative leader with strong managerial skills who is well versed in health policy, has experience with federal health programs, and seeks to make a positive impact on the public's health.

The mission of IOM is to serve as adviser to the nation to improve health. Working outside the framework of government, the IOM provides scientific, evidence-based information and advice to policy makers, professionals, the private sector, and the public.

The successful candidate will work closely with the IOM President and will provide intellectual, operational and financial leadership for IOM's programs and staff in accordance with the policy guidance of the IOM President.

This position requires an MD, a PhD in a related field, or equivalent knowledge relevant to this position, and 10 years of related professional experience, including 5 years in a supervisory capacity.

For more information about this challenging career opportunity and to apply, please visit our website at <http://national-academies.org> - click on Careers - select Current Opportunities - and search openings by Department - Institute of Medicine.

Established in 1970 under the charter of the National Academy of Sciences, the Institute of Medicine provides independent, objective, evidence-based advice to policymakers, health professionals, the private sector, and the public. The National Academy of Sciences, National Academy of Engineering, Institute of Medicine, and National Research Council make up the National Academies. Headquartered in Washington, DC. EOE/M/F/D/V.

national-academies.org



FACULTY POSITION IN COMPUTATIONAL BIOLOGY AND DATA SCIENCE AT THE BOSTON COLLEGE BIOLOGY DEPARTMENT

The Boston College Biology Department seeks outstanding candidates for a tenure-track faculty position in the area of Computational Biology and Data Science. Applicants are sought at the Assistant Professor level; however, exceptionally strong candidates will be considered at the Associate Professor level. The university provides competitive start-up funds and research space with the expectation that the successful candidate will establish, or bring to the university, a vigorous, funded research program. The successful candidate will join an active computational biology team including four faculty members, numerous postdocs and doctoral students, professional staff, and substantial computational hardware resources. Current strengths in our computational group are in sequencing software development, genomic variation analysis, microbial systems biology, and in RNA structure and function.

The aim of this search is to recruit a computational scientist whose research is directed at biomedical "big data" analysis tool development e.g. algorithms for mining, accessing, integrating, and/or visualizing DNA sequencing, proteomic, image, or other large-scale biomedical data, with application areas in basic biological or medical research. Candidates working in areas with clear relevance to human health (e.g. human disease genetics, cancer or pathogen research), and those who combine wet-lab experimentation with computational algorithm development, will be given priority. Special consideration will be given to candidates whose research program synergizes with current faculty interests in Infection/Immunity and in Developmental/Cellular Biology (see <http://www.bc.edu/biology> for profiles of the Biology Department's current research programs).

Applicants should submit a *curriculum vitae*, a statement of research and teaching plans, and arrange for three letters of reference. All application materials should be submitted via Interfolio at <http://interfolio.com/23020>. Review of applications will begin on **October 1, 2013**, and continue until the position is filled.

Boston College is an Affirmative Action, Equal Opportunity Employer. Women and minority candidates are especially encouraged to apply.



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, Science Careers offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, Science Careers has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/sciencecareers



POSITIONS OPEN

TENURE TRACK FACULTY POSITIONS in The Field of "Gene Expression and Signaling" Department of Biochemistry and Molecular Biology

The Department of Biochemistry and Molecular Biology (website: <http://www.bmb.msu.edu>) at Michigan State University (MSU) seeks to recruit two outstanding tenure system ASSISTANT/ASSOCIATE PROFESSORS. The individuals will employ innovative and multidisciplinary approaches to address central questions in gene expression and signaling in development and disease. Successful candidates will contribute to the highly collaborative research environment on campus. The position is intended to integrate with existing research in one or more of the following groups: Gene Expression in Development and Disease, Molecular Metabolism and Disease, Mitochondrial Science and Medicine, and Bio/computational Evolution in Action Consortium; and/or with ongoing cancer and neuroscience research.

Review of application materials will begin on November 11, 2013, and continue until suitable candidates are identified. Applicants should upload a single PDF file with cover letter, curriculum vitae, up to three pages of research interests and future directions, and a one-page statement of teaching philosophy to website: <https://jobs.msu.edu> (position #6878). Three to five letters of reference should be sent to e-mail: gesfacultysearch@cns.msu.edu. Questions about the position can be addressed to the Chair of the Search Committee, Min-Hao Kuo at (e-mail: gesfacultysearch@cns.msu.edu).

MSU is an Affirmative Action/Equal Opportunity Employer and is committed to achieving excellence through a diverse workforce and inclusive culture that encourages all people to reach their full potential. The University actively encourages applications and/or nominations of women, persons of color, veterans, and persons with disabilities. MSU is committed to providing a work environment that supports employees' work and personal life, and offers employment assistance to the spouse or partner of candidates for faculty and academic staff positions.

MARINE AND COASTAL SCIENCES

The Department of Ecology and Evolutionary Biology at Tulane University seeks a full-time, non-tenure-track PROFESSOR OF THE PRACTICE (PoP) beginning fall 2014. The PoP will administer marine biology minors and teach introductory marine biology and other courses in marine and coastal sciences. An earned doctorate in biological sciences or other appropriate field is required. We seek an exceptional individual with a commitment to excellence in undergraduate education. PoPs are appointed for initial three-year, renewable terms. More information about the position can be found at website: <http://tulane.edu/sse/eebio/about/pop.cfm>. To apply, submit curriculum vitae, statement of teaching philosophy and proposed classes, description of scholarly and teaching interests and experience, and the names and addresses of three references electronically to e-mail: ecolevol@tulane.edu. Review of applications will begin November 15, 2013, and the position will remain open until filled. Tulane University is an Affirmative Action/Equal Employment Opportunity/ADA Employer committed to excellence through diversity. All eligible candidates are encouraged to apply. This position is subject to final budgetary approval.

TENURE-TRACK FACULTY POSITIONS in Bioarchaeology and Scientific Ecological Anthropology

The University of California, Santa Barbara (UCSB), Department of Anthropology, seeks a Bioarchaeologist, and a Scientific Ecological Anthropologist for tenure-track positions at the ASSISTANT PROFESSOR level. Submit application and materials by November 15, 2013 for the first position, and by the same date for primary consideration for the second position. For more details and to apply go to website: <https://recruit.ap.ucsb.edu>. UCSB is an Equal Opportunity/Affirmative Action Employer.

Download your free copy today.

ScienceCareers.org/booklets



From technology specialists to patent attorneys to policy advisers, learn more about the types of careers that scientists can pursue and the skills needed in order to succeed in nonresearch careers.

Science Careers

From the journal Science

